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Investigating the Role of Coyotes, Canis Latrans, in the Spread of Parasites and Arthropod-Borne Diseases in Georgia, USA.

Ansleigh Banks

INVESTIGATING THE ROLE OF COYOTES, *CANIS LATRANS*, IN THE SPREAD OF PARASITES AND ARTHROPOD-BORNE DISEASES IN GEORGIA, USA.

by

ANSLEIGH SARAH BANKS

(Under the Direction of William S. Irby.)

ABSTRACT

In order to analyze the role of coyotes, *Canis latrans,* as hosts of parasites and arthropod-borne diseases in the state of Georgia, USA, 38 coyotes representing 18 counties and multiple geographic regions of the state were dissected and analyzed for the presence of *Dirofilaria immitis,* intestinal parasites, ectoparasites, and tick-borne illnesses. Mosquitoes from the locations which the coyotes originated were trapped and analyzed for the presence of *D. immitis* larvae. In compliance with IACUC guidelines, coyotes were obtained from individuals who had previously hunted or trapped the coyotes for leisure. Parasites were identified based on morphological features with the use of dichotomous and pictorial keys. A total of 1086 parasites were collected: 215 intestinal nematodes, 116 cestodes, 1 acanthocephalan, 359 filarial nematodes, 128 ticks, 10 fleas, and 257 lice. Of the 38 coyotes examined, 21 had adult *D. immitis* present, 30 had adult parasites or ova present, and 19 had ticks present. Trends in prevalence and burden of parasites among different regions of the state, and different sexes, ages, and sizes of coyotes were analyzed. Three specimens had no parasite of any form present (7.89%), while 11 of the specimens were found to be affected by heartworms, intestinal parasites, and ectoparasites (28.94%). Of 30 specimens analyzed for exposure to tick-borne illness with the SNAP 4dx ELISA test, 10 were found to have been exposed to *Ehrlichia canis* or *Ehrlichia ewingii* (33%), and 1 was found to have been exposed to *Borrelia burgdorferi* (3.33%). Gravid trapping of mosquitoes yielded specimens belonging to 7 species which are vectors of *D. immitis*. Two mosquitoes were suspected to be infected with *D. immitis* larvae; however, molecular detection methods must be performed to confirm this. The results of this study provide a more diverse record of parasitism and arthropod-borne diseases in Georgia's coyote population as samples were taken from as many different locations throughout the state as possible. This study found coyotes to be wildlife hosts and modes of transportation for parasites which can negatively affect the health of both humans and domestic animals.

INDEX WORDS: Coyote, *Canis latrans*, Georgia, Parasites, *Dirofilaria immitis*, Heartworm, Cestode, Tapeworm, Nematode, Hookworm, Whipworm, Acanthocephalan, Tick, *Ehrlichia*, Lyme disease, Flea, Lice, Mosquito

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ANSLEIGH BANKS

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ANSLEIGH S. BANKS

Major Professor:	William Irby
Committee:	Lance Durden
	Stephen Greiman

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DEDICATION

I would like to dedicate this thesis to a multitude of people who have helped me to complete this project and earn my master's degree.

This project would not have been possible were it not for my thesis committee. First and foremost, thank you to Dr. Irby for being a great undergraduate advisor, and accepting me as a graduate student when I decided to come back to school. I would not have finished this project were it not for his helpfulness and guidance. Also, a huge thank you to Dr. Durden and Dr. Greiman for their support, encouragement, expertise, and help with data analysis.

The wonderful staff and faculty in the Georgia Southern University Department of Biology also helped tremendously with my graduate career. To all the professors who helped me to learn and excel, thank you! Thank you to Dr. Checo for his help as the head of the graduate program, and thank you to Janee Cardell and Dyana Van Tassel for all their help making our lives as graduate students easier. Thank you to Michelle Tremblay and Dr. Ndaluka for "teaching me how to teach", and also to my students throughout my time as a graduate TA at GSU for helping me to become better at teaching.

Thank you to the amazing coyote hunters who went out of their way to preserve specimen for me to utilize for this project and helped me to locate good sites for mosquito trapping, and to the landowners who allowed me to trap mosquitoes on their land.

My amazing friends and officemates Bonnie Cobb, Mattie Whitesell, Anna Wagner, Nicole Kleinas, T.J. Odom, Shawna Defreitas, Tyler Follman, Debbi Albanese, Katelyn Cranmer, and many more were always there for me when I needed a shoulder to cry on or a couch to sleep on, and I could not be more thankful for their presence in my life. Also, a huge thank you to my boyfriend whom I ironically met through this project while networking to find coyote hunters. He has been there for me whenever I needed someone to talk to, moral support during field dissections, and 4wd to go place mosquito traps in the middle of a muddy cow pasture.

Finally, thank you to my family for their continual love and support. I would not be where I am today if it weren't for my parents support and guidance. For helping me throughout my college career, helping me move in and out of multiple apartments during grad school, and helping me learn how to be a responsible adult, thank you. And also, thank you for helping me with some dirty, smelly field dissections for this project!

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A huge thank you to my parents and friends who assisted me with recording data and labeling storage containers for organs during some very smelly field dissections! Thank you to all my friends, classmates, and officemates for their support both in and outside of school - we truly have a great family in the Biology department!

While many individuals have helped me with my research and data analysis, this project would not have been possible were it not for the hard work of the hunters and trappers who obtained these coyotes and donated them to my project. I am eternally grateful to multiple individual trappers (names withheld for privacy), the Georgia Predator Hunting Association and its members, and the landowners who allowed me to access their properties to trap mosquitoes where the coyotes were trapped.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND DEMOGRAPHICS OF SAMPLE POPULATION

Purpose of the Study

This study aimed to evaluate the burden of endoparasites, ectoparasites, and their associated diseases in coyotes with the intent of providing results that can help to better understand the risk of parasite and arthropod-borne disease transmission that coyotes pose to domestic dogs and humans in the state of Georgia. In this study, hunted and trapped coyote carcasses from a variety of geographic locations throughout the state of Georgia were examined for the presence of ectoparasites, enteric parasites and canine heartworm disease and exposure to 3 tick-borne bacterial pathogens (*Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi)*. Trapping and dissection of mosquitoes to determine which species might serve as local vectors of *D. immitis* was also performed in locations proximate to where coyotes originated.

How This Study Is Original

While many previous studies on coyotes in Georgia or neighboring states focused on specimens in specific land management areas, this study aimed to assess coyotes from different geographic regions throughout the state and provide more comprehensive knowledge on the parasites and arthropod-borne diseases currently affecting Georgia's coyote population.

Coyotes in Georgia

Coyotes, *Canis latrans*, are a wild canid species which are heavily populated throughout a majority of regions in North and Central America. They were first introduced to the state of Georgia in the 1950's through illegal transport by hunters who used them for training hunting dogs, and are considered a non-native, invasive nuisance species (Nesmith, 2017). Georgia's coyote population has been divided into two categories based on tracking and monitoring: resident coyotes numbering approximately 250,000; and transient coyotes with an estimated population of 90,000 (Blankenship, 2019). Resident coyotes are classified as those which continually occupy an area of land no greater than 10 square miles, whereas transient coyotes travel distances as great as 100 miles at a time (Blankenship, 2019). GPS tracking of coyote populations in the Piedmont region of Georgia showed that coyotes vary quite significantly in their home-range sizes, from 1.8 to 45 square miles (Hickman, 2014).

Known to fill an ecological niche as primarily carnivorous mesopredators which typically consume small mammals such as rodents, rabbits, and raccoons, coyotes are recognized by the Georgia Department of Natural Resources as an effective control species for rodent populations (GA DNR, 2017). In areas where wolves are absent, most often due to extirpation, coyotes are known to assume the role of apex predators (Berger and Conner, 2008). Analysis of coyote fecal matter, known as scat, collected from various regions throughout the state provides evidence that coyotes are capable and currently acting as an apex predator in Georgia. Remains of two large species: the Wild Boar, *Sus scrofa*, and the White-tailed Deer, *Odocoileus virginianus*, have been identified in coyote scat from Cumberland Island, Georgia (Whitaker et al. 2015). Scat collected from the Joseph W. Jones Ecological Research Center in Southwest Georgia, an area representative of Georgia's Long-Leaf Pine Ecosystem, was analyzed and indicated that various species of rodents, rabbits, and birds, White- tailed Deer, and vegetation primarily made up the coyote's diet, with the occasional presence of prey classified as mesoanimals including armadillos- *Dasypus novemcinctus*, Striped skunks- *Mephitis mephitis*, raccoons- *Procyon lotor*, Virginia opossums-

Didelphis viginiana, bobcats- *Lynx rufus*, and grey foxes- *Urocyon cineroargenteus* (Cherry et al. 2016).

Due to their classification as an invasive species in the state of Georgia, coyotes are classified by the Georgia Department of Natural Resources as a nuisance species with no hunting season or limit enforced, and no restrictions placed on the use of live foot-hold traps (GA DNR, 2017). The effect of coyote predation on the population of White-tailed Deer, an important game species in the state of Georgia, has been under investigation, with the goal of determining effective measures for controlling coyote populations to preserve White-tailed deer populations (Gulsby et al., 2015). The effects of coyote predation on White-tailed deer fawns in Georgia has been under investigation in recent years and revealed that coyotes play a large role in fawn mortality, accounting for the proven deaths of 37% (and suspected 80%) of monitored fawns at the Savannah River Site along the South Carolina/ Georgia border (Kilgo et al, 2012). Additional studies in the same study area found that in the deer/coyote prey/ predator relationship, the number of coyotes present is the more important factor in fawn mortality rates rather than the number of deer present (Gulsby et al, 2015).

Beginning in March 2017, the Georgia Department of Natural Resources began a program known as the Coyote Challenge, with the goal of encouraging outdoorsmen and landowners to hunt coyotes from March to August, as their research indicated that it is during these months that coyotes have the greatest effect on native wildlife. During the inaugural year, 2017, a total of 191 coyotes were killed and the next season, 2018, had greater results with a total of 431 coyotes killed (GA DNR 2017; GA DNR, 2018). Hunters were allowed to submit 5 coyote carcasses per month of the contest to their local DNR office, with each submission entering their name in a raffle for a lifetime hunting license. As of August 2019, there is no evidence available on the DNR's website that the Coyote Challenge has returned for a third year, possibly because it gained media attention as a "bounty" program by animal activist groups such as the Atlanta Coyote Project. While Atlanta Coyote Project representative's labeling of the program as a bounty was innacurate, they did bring

up an important point that the culling efforts were likely to have little effect on the coyote's population size.

Coyotes are both a socially and genetically monogamous species, meaning that as a whole, they mate with one individual of the opposite sex for the entirety of their lifetime, commonly known as mated pairs (Hennessey et al, 2012), with these mated pairs presiding over a given territory (Gese, 2001). Breeding is seasonal, occurring in the range of January to March (Carlson and Gese, 2008), followed by a gestation period of 60-63 days (Kennelly et al, 1977). Whelping of pups typically occurs between March and May (Kennelly et al, 1977). Evidence exists that coyotes can respond to exploitation by culling through increased fecundity by breeding of yearlings (1-2 years of age), which do not breed at as high a rate as adults do, e.g., the yearling breeding rate of a coyote population in Colorado increased from 0% prior to exploitation to 20% following 2 years of exploitation (Gese, 2005). While an average increase in litter size was also observed in years following exploitation (removal), the effects of increased availability of prey indicates that the response to exploitation was not solely responsible for the increased fecundity observed (Gese, 2005). Research conducted at the Savannah River Site along the South Carolina/ Georgia border revealed that attempts to control coyote populations had limited and inconsistent success on White-tailed deer fawn survival (Kilgo et al, 2014; Gulsby et al, 2015), thought to be due to the coyote's ability to respond to population loss through increased litter size. Related studies on coyote reproduction in response to pressure by trapping and subsequent euthanasia of coyotes also conducted at the Savannah River Site indicated that a seasonal population decline occurred during the three years of trapping; however, the population consistently returned to its pre-trapping size in less than a year, with a shift towards a younger population (Kilgo et al, 2017). Additionally, transient coyotes mixed with resident populations in search of a permanent territory (Chamberlain et al, 2000), which helped to maintain and increase population sizes, making population control even more difficult.

Because there are no regulations on season or a limit on the number of coyotes an individual hunter is allowed to kill or trap within a season, and because of the likely significant role they play in vector-borne disease, coyotes were chosen for this project to investigate parasitism and vector-borne diseases in wild canids. The domestic dog, *Canis lupus familiaris*, and Coyote, *Canis latrans,* are closely related members of the family Canidae separated only by the Grey Wolf, *Canis lupus*, and are therefore likely to be infected and or infested by species of parasites and vector-borne diseases of veterinary importance to companion animals, particularly domestic dogs. In addition, some of these parasites and vector-borne diseases are zoonotic and have the ability to affect humans.

MATERIALS AND METHODS

Source of Specimens

In accordance with IACUC exemption guidelines, no animals were killed or harmed for the purpose of this project; all coyotes used in this project were previously killed for independent culling activities and their carcasses were donated. Through research on online outdoor forums and predator hunting websites, connections were made with hunters and trappers who are active in various regions throughout the state of Georgia (located in the Southeastern region of the United States). A total of 38 coyotes (19 Males, 19 Females) were examined. Samples were taken in 2018 and 2019 from the Georgia Predator Association's "Coyote Classic" 48-hour hunting competition which occurred February 23-25 2018 and February 8-10 2019 as well as from individual hunters and trappers from December 2017-March 2019. Coyotes used in this study came from the following Georgia counties: Atkinson, Bartow, Cherokee, Dooly, Dougherty, Franklin, Gordon, Gwinnett, Jones, Pickens, Sumter, Terrell, Treutlen, Troup, Twiggs, Wilkes, and Wheeler, as shown in Figure 1.3. The counties in which the coyotes were hunted were grouped into geographic and Department of Natural Resources (DNR) management regions. The geographic regions and the counties which they include are listed in Table 1.1 and illustrated in Figure 1.1, and DNR regions and their respective counties listed in Table 1.2 and illustrated in Figure 1.2. A map indicating locations sampled was created using the starred locations feature on the Google Maps website (Figure 1.3)

Table 1.1: Geographical regions of Georgia and the sampled counties which they include.

Geographical Region	Counties
Ridge and Valley	Bartow, Gordon
Piedmont	Cherokee, Franklin, Gwinnett, Jones, Pickens, Troup, Wilkes
Upper Coastal Plains	Dooly, Dougherty, Sumter, Terrell, Treutlen, Twiggs, Wheeler
Lower Coastal Plains	Atkinson, Toombs

Figure 1.1: Map of geographic regions of Georgia.

Table 1.2: Georgia Department of Natural Resources Management regions and the sampled counties which they include.

Geographical Region	Counties
Northwest	Bartow, Cherokee, Gordon, Pickens,
Northeast	Franklin, Gwinnett
West Central	Jones, Troup, Twiggs
East Central	Wilkes
South Central	Atkinson, Toombs, Treutlen, Wheeler
Southwest	Dooly, Dougherty, Sumter, Terrell

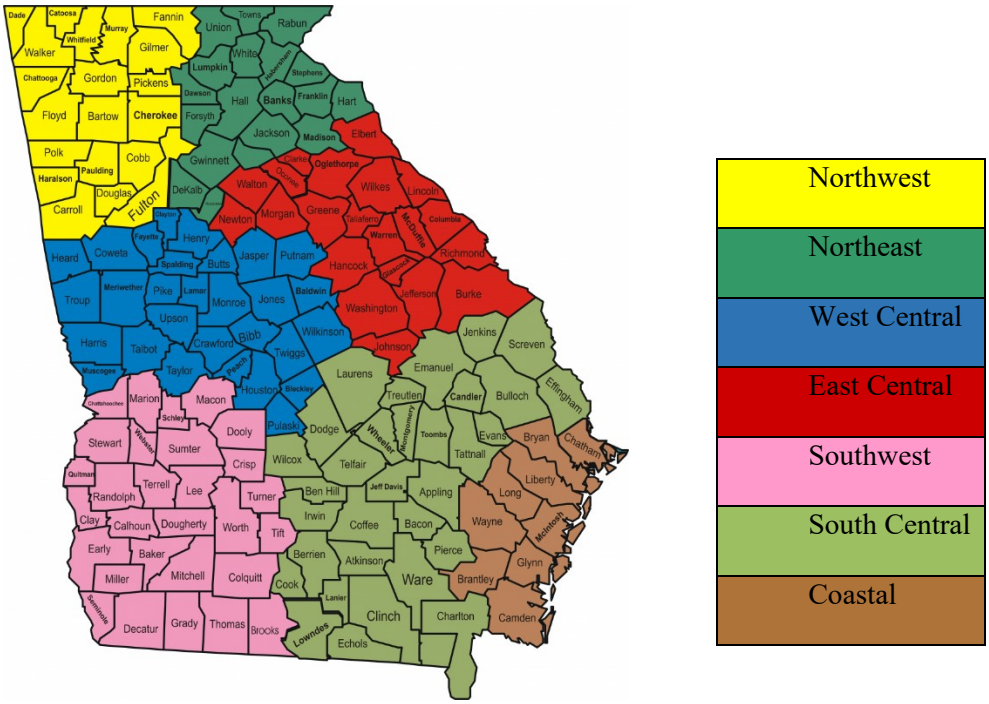

Figure 1.2: Map of DNR management areas of Georgia.

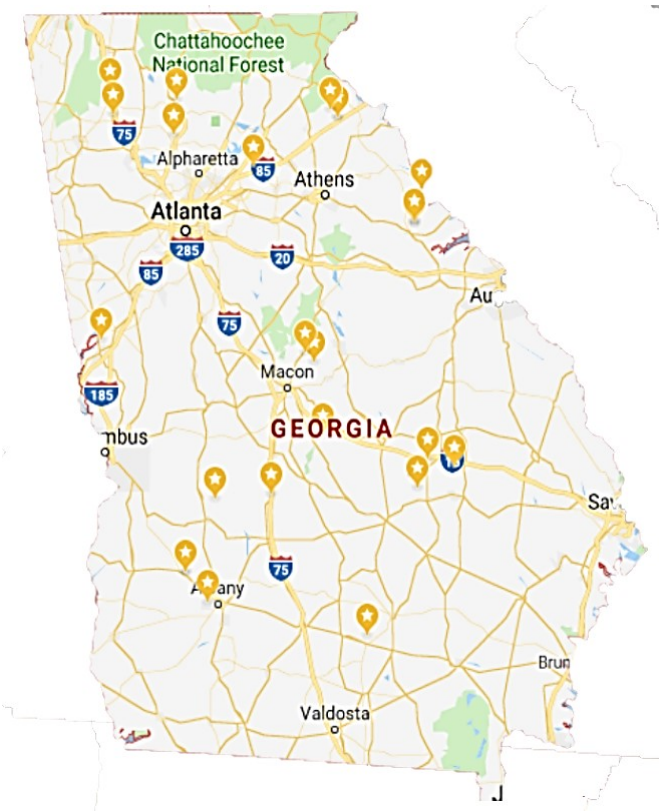

Figure 1.3: Map of locations which sampled coyotes originated from.

Safety Precautions

Personal protective gear worn during coyote dissections included nitrile gloves, safety goggles, a protective mask over the nose/mouth, a protective gown over clothing, waterproof close-toed shoes, and clothing to cover exposed skin. The same personal protective gear was worn while dissecting internal organs in the laboratory, in addition to performing dissections of organs behind a glass barrier in a fume hood. Pre-exposure rabies vaccinations were received before beginning the project.

Measurement of Demographical Data

Prior to dissection, sex and approximate age of each coyote were recorded. Sex was identified by external genitalia and age was estimated using criteria including general body size, shade of teeth, and amount of dental tartar (similar to methods used by veterinarians to estimate age of

domestic dogs). A pictorial guide to estimating coyote age by teeth created by the Atlanta Coyote Project aided in estimating age (Figure 1.4). The 7 coyotes collected at the February 2019 "Coyote Challenge" hunting competition were weighed in pounds with a hanging scale by contest officials, and 1 coyote was weighed opportunistically on a floor scale at a veterinary office.

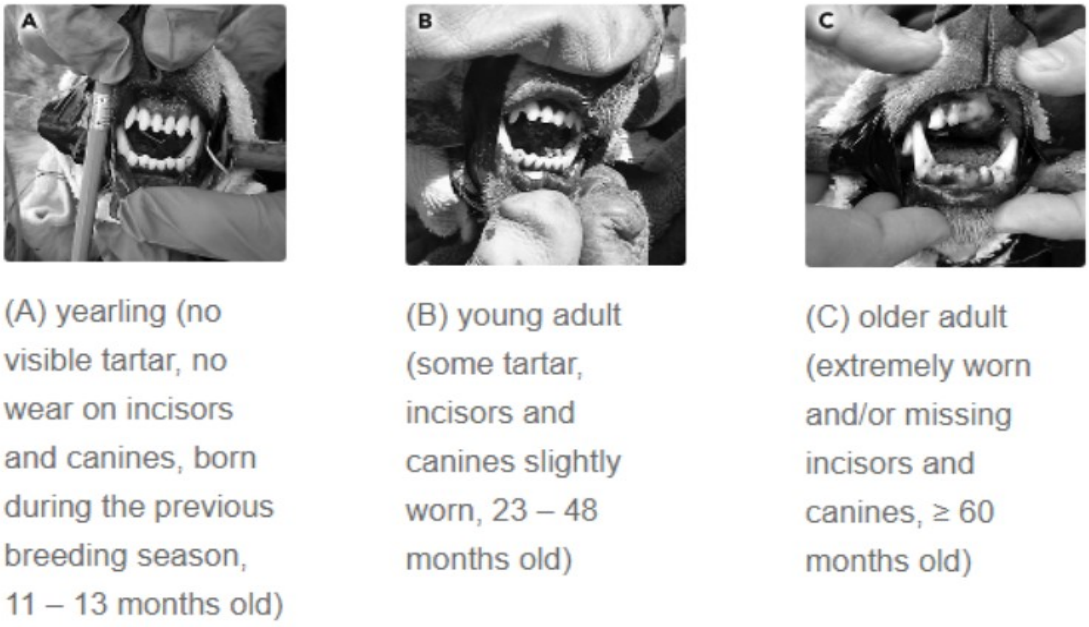

Figure 1.4: Visual guide to age estimation and determination based on teeth created by Atlanta Coyote Project through tracking and photographing throughout coyote's lifespan.

Statistical Analysis of Demographical Data

A t-test was used to analyze possible correlation between sex and approximate age of coyotes tested. The effect of sex on both estimated age and weight was tested with analysis of variance testing (ANOVA), with Levene's test for equal variances amongst groups. ANOVA was also used to test for differences in approximate weight between region (both DNR management and geographic) and contingency tables were used to analyze differences in approximate age range distribution between regions.

Disposal Following Dissection

At the Georgia Predator Hunting Association's events, coyotes were disposed of in a group burial pit. For coyotes dissected in the animal facility field house, all carcass materials were double-bagged and disposed of at the landfill and transfer station (911 N Main St, Statesboro, GA 30458). All surfaces and instruments were soaked then manually cleaned with a solution of 10% bleach, followed by a solution of Lysol.

Figure 1.5: Coyotes from 2019 "Coyote Classic" hunting Competition.

RESULTS AND DISCUSSION:

Demographics of Sample Population

A total of 38 coyotes (19 males, 19 females) were examined in this study. Of the specimen collected, 26 were killed by hunting, and 12 by catching in a foot-hold trap and dispatched by a shot to the head. Four of Georgia's geographic regions, and six of its DNR management regions were represented (Tables 1.3 & 1.4). The majority of the coyotes examined were estimated to be young adults 1-2 (n=24) and 2-3 (n=10) years of age, with smaller amounts of juveniles less than 1 year of age (n=2) and middle-aged adults thought to be over 3 years of age (n=2) (Figure 1.6). Approximate weight was not affected by sex (Levene p=0.508), but did vary significantly based on approximate age (n=38, df=3, p>F= 0.0005), with weight increasing with age (Figure 1.7). Neither DNR management region (n=38, df=5, p>F=0.5310), nor geographic region (n=38, df=3, p>F=0.8228) of origin had an effect on the approximate weight of coyotes. Approximate age of coyotes was also not affected by DNR region (n=38, df=15, $p>X^2=0.2070$) (Figure 1.8), or geographic region (n=38, df=, $p>X^2=0.0819$) (Figure 1.9).

Table 1.3: Number of coyotes examined per geographic region.

Geographic Region	Number of Coyotes
Ridge and Valley	2
Piedmont	19
Upper Coastal Plains	13
Lower Coastal Plains	4

Table 1.4: Number of coyotes examined per DNR management region.

DNR Region	Number of Coyotes
Northeast	6
Northwest	6
East Central	4
West Central	7
South Central	8
Southwest	7

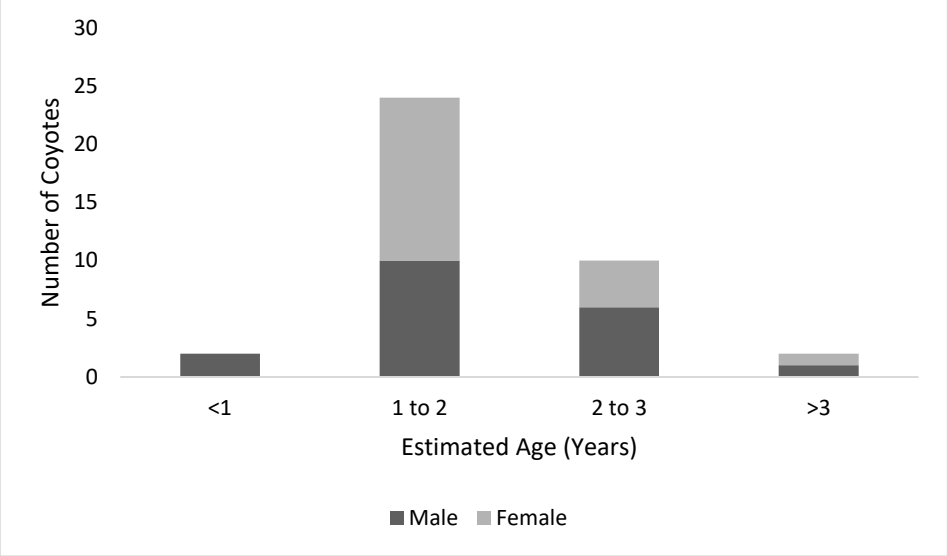

Figure 1.6: Estimated age and sex distribution among coyotes examined.

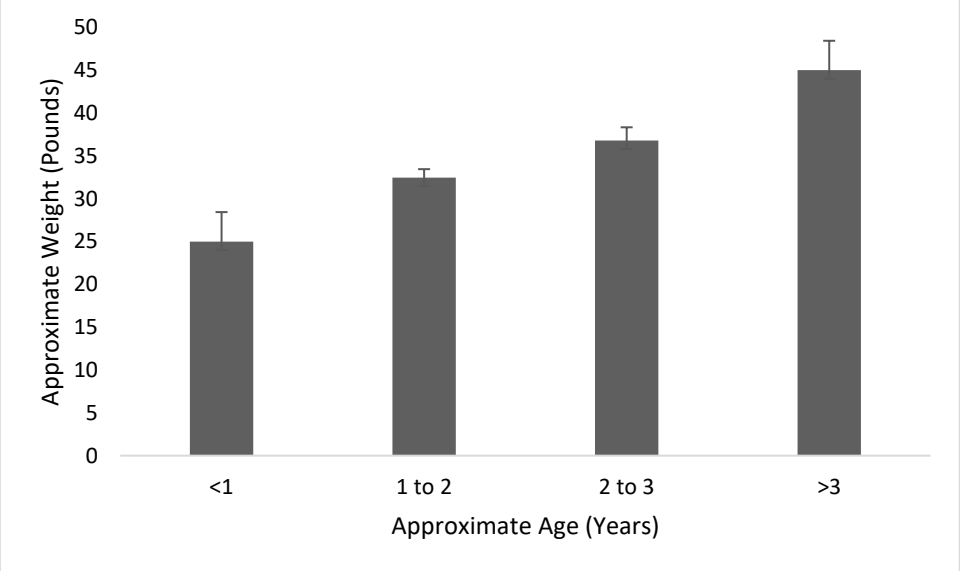

Figure 1.7: Variation in approximate weight based on approximate age.

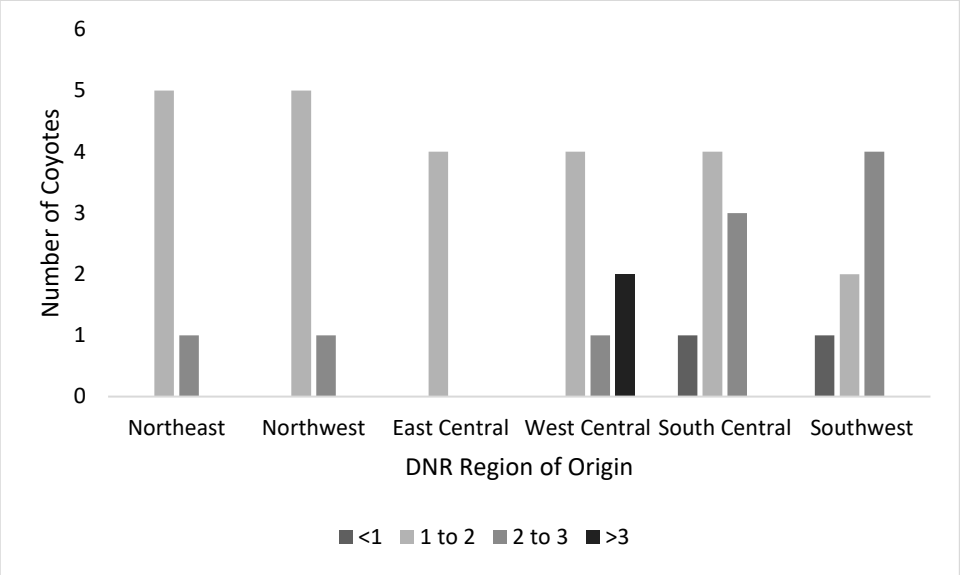

Figure 1.8: Approximate age of coyotes examined based on DNR management region.

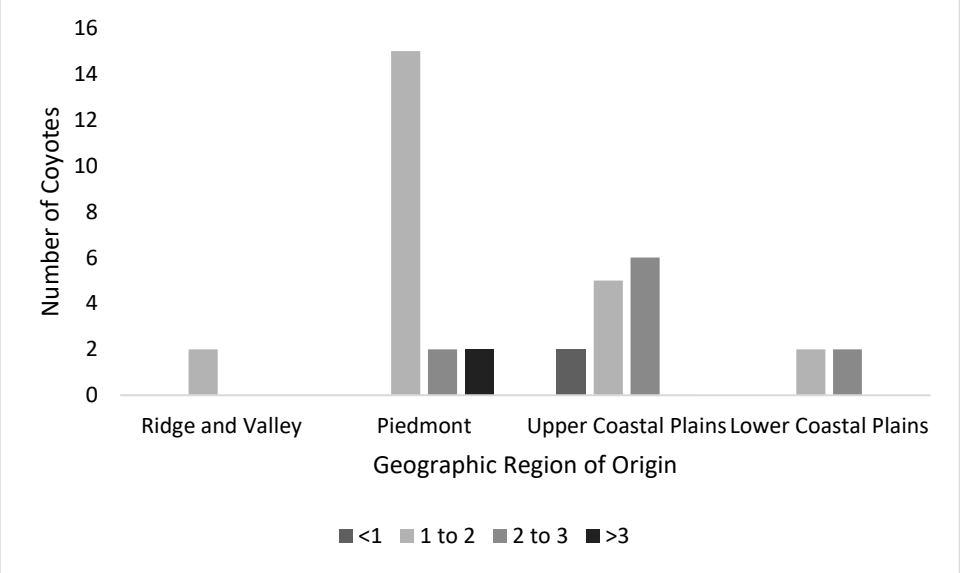

Figure 1.9: Approximate age of coyotes examined based on geographic region.

Partnering with the Georgia Predator Hunting Association to collect specimen at the "Coyote Challenge" in both 2018 and 2019 allowed for specimens from a large variety of regions to be collected without physically having to travel to these locations as all coyotes were turned in at the weigh-in checkpoint in Fort Valley, located in central Georgia. While this helped tremendously with the cost and time associated with travel to obtain specimen, there were also disadvantages associated with this method of sampling. The post-mortem interval ranged from 6-40 hours, with the integrity of specimens declining as the post-mortem interval increased. Because of the rapidly declining condition of specimens (bloating, flies laying eggs in orifices, friability of tissues) and lack of facilities to preserve whole coyotes in a freezer until dissection could be performed in the laboratory, the number of specimens collected was limited to those that could be dissected in the field in the allotted 2-3 hours after weigh-in before all coyotes were placed in a group burial pit. Due to the nature of the competition allowing hunted coyotes only, the majority of specimens were shot in the thorax and/or abdomen as these surface regions of the body are larger and easier to hit than the head. Excessive trauma to the intestines or heart (organs essential to this study) disqualified many specimens from use. The 12 specimens which were obtained from trappers contained bullet wounds confined to the cranial region, and were bagged and preserved on ice or frozen immediately post-mortem, resulting in better-preserved specimens with ectoparasites retained.

Both geographic and DNR management regions were used to compare data in this study due to the characteristics of each grouping. While the geographic regions are lesser in number and more descriptive of geographic features of the land, the variability in size of the 5 regions (Figure 1.1) resulted in the majority of the coyotes (n=32) being classified from the Piedmont and Upper Coastal Plains region (Table 1.3), causing a disproportionate clustering of data. Grouping counties of origin by the 7 DNR management regions did lessen the sample size per region, but helped to equalize the number of samples per region (Table 1.4), and the names of the regions are more indicative of the general area of the state which coyotes originated from. Not all regions were

sampled: the Northeastern “Blue Ridge Mountains” geographic region was not included; nor was the Southeastern “Coastal” DNR management region.

The approximate ages of coyotes from the study were more evenly dispersed than a previous post-mortem study of Georgia coyotes where 15 of 31 total specimens were less than 1 year of age (Gates et al, 2014); however, with only 2 land management sites sampled, that study had a much smaller available population and therefore may have possibly targeted packs still containing populations of juveniles. Based on age estimation by dental tartar and wear, no coyotes over the age of 3 to 4 years were examined in this study, which also differs from the previously mentioned study, with several coyotes listed as 4,5, and 6 years of age (Gates et al, 2014). This may also be explained by hunting techniques utilized such as targeted hunting on a pack of coyotes, or failure to accurately estimate age by experimenters from either study. The 14 coyotes collected at the February 2018 “Coyote Challenge” were weighed with a hanging scale, however the data sheets were misplaced by contest officials. For any coyote for which an official weight was not recorded due to loss of data or lack of access to a scale, weight was estimated to a 5-pound range by the dissector’s judgement. The dissector had 5 years of experience lifting domestic canines at a veterinary clinic and provided estimates within a 6-pound range of official weights recorded by hanging scale for specimen collected at the “Coyote Challenge” in 2019, indicating likely accuracy of estimated weights.

A logical trend observed was the increase in average estimated weight as the estimated age increased. The two coyotes classified as juveniles examined in this study were both approximately 25 pounds, whereas 1 to 2 year olds (young adults) were an average of 32 pounds, 2 to 3 year olds (young adults) averaged 36 pounds, and middle-aged adults between the age of 3 and 5 were both approximately 45 pounds. This positive correlation between age and weight was also observed in a post-mortem survey of coyotes from Georgia (Gates et al, 2014).

The lack of correlation between both DNR management and geographic regions in relation to both approximate age and weight in the sampled coyote population was not unexpected, as

sampling was conducted randomly. The even distribution of approximate age throughout the various regions of the state is likely to strengthen the results of the study due to the evenly distributed sample population. Collecting a high percentage of juvenile or older adults from a region is likely to skew data as any older organism has had a longer amount of time to be exposed to parasites, and is therefore more likely to have an infection than a younger specimen. The results of parasites found upon examination and dissection of the coyotes as well as results of trapping and dissection of mosquitoes (vector of *Dirofilaria immitis*) can be found in subsequent chapters (see table of contents).

CHAPTER 2

CANINE HEARTWORM DISEASE

Canine Heartworm Disease in the United States

Canine Heartworm Disease is caused by the parasitic nematode *Dirofilaria immitis* (the Canine Heartworm) and spread through the bite of an infected mosquito. This disease is widespread in the Southeastern United States, where veterinary clinics report the highest prevalence of Canine Heartworm Disease cases in the continental United States (Figure 2.1). Heartworm tests results from individual veterinary clinics reported back to the Idexx test manufacturer and analyzed over a 7- year period indicated that 3.6% of tested Southeastern dogs were heartworm positive, a much higher number than the Northeast (0.6%), Midwest (0.8%), and West (1.2%) (Bowman et al, 2009).

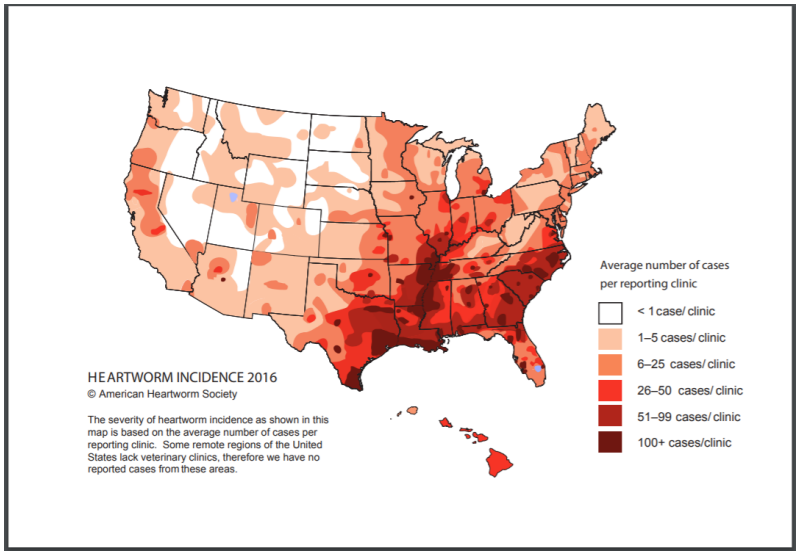

Figure 2.1: Incidence map of Canine Heartworm Disease cases reported to the American Heartworm Society, 2016.

According to previous studies, over 25 species of mosquitoes belonging to the genera *Aedes, Anopheles, Culex, and Psorophora* serve as competent vectors of *D. immitis*, most of which can be found in the Southeastern United States (Ledesma and Harrington, 2011). DNA analysis of

mosquitoes trapped in 7 counties in Western Georgia (Clayton, Cobb, Dougherty, Henry, Muscogee, Sumter, and Talbot) found *Aedes albopictus, Aedes vexans, Anopheles punctipennis,* and *Anopheles crucians* were caught in the highest numbers in CDC light traps and gravid traps, and infected with *D. immitis* L3 larvae (Licitra et al, 2010). Although many mosquitoes are competent vectors, the presence of certain species including *Aedes canadensis, Aedes trivittatus, Anopheles punctipennis, Anopheles quadrimaculatus*, and *Culex quinquefasciatus,* (all of which are present throughout Georgia with the exception of *Aedes trivittatus* which is absent from the southern-most part of the state) have been linked to high heartworm prevalence in canine populations (Wang et al, 2014).

Mosquitoes act as both the vector and intermediate host while various species of canids act as definitive hosts. While blood-feeding on an infested canine, mosquitoes ingest microfilariae, a motile embryo of *D. immitis*. The microfilariae mature and undergo three larval stages: L1, L2, and L3 in the Malpighian tubules of the mosquito. The maturation process is affected by temperature and varies in time from 11 to 26 days post-infection before *D. immitis* migrates to the mosquito's salivary glands (Ledesma and Harrington, 2015). *D. immitis* can then be transmitted through the proboscis of the infested mosquito when it deposits saliva containing analgesics and anticoagulants onto the skin as it takes a blood meal from another canine (Mullen and Durden Ch. 14, 2009). The L3 larvae enter the body through the wound left from the mosquito's blood meal and continue to develop in the subcutaneous tissue of the host. The begin a molt to the L4 stage after a period of time ranging from 3-12 days post-infection and begin migration towards the thoracic region of the host via the peripheral veins. The *D. immitis* lodge in the pulmonary artery, where they mature into the adult life stage and begin sexually reproducing and release microfilariae into the bloodstream after approximately 6 months after infection by the mosquito (Venco et al, 2015). This infested canine host is now microfilaremic, circulating microfilariae in its peripheral bloodstream, where they can be ingested by a mosquito, and transmitted to another definitive host.

Definitive hosts of Dirofilaria immitis

Many species of canids are capable of becoming definitive hosts of *D. immitis*, including Domestic dogs- *Canis lupus familiaris*, Coyotes- *Canis latrans*, Gray Wolves- *Canis lupus*, Red Wolves- *Canis rufus*, and Red Foxes- *Vulpes vulpes*. On rare occasions, some members of the suborder Caniformia such as Ferrets- *Mustela putorius futo*, and Sea Lions- *Zalophus californianus* have been reported to serve as hosts for canine heartworm disease (American Heartworm Society, 2018). Domestic cats- *Felis catus domesticus* also act as incidental hosts for *D. immitis* although the incidence rate of adult heartworm infections in felines is much lower than that recorded in canines. While cats receive similar exposure to infected mosquito bites, the feline host's inflammatory response to juvenile heartworms reaching the lungs tends to eliminate the parasite before it can lodge in the pulmonary artery (Venco et al 2015). Heartworms that do survive to the adult stage in feline hosts are also present in smaller numbers than in canine hosts, with most feline infections consisting of 6 or less adult worms (Venco et al, 2015). However, due to the smaller size of the feline heart compared to a canine heart, feline hosts may potentially be more susceptible to death from the burden of an intra-cardiac parasite present in smaller numbers, thus preventing larger infections from occurring. On rare occasions, humans may become infested with *D. immitis;* however, the parasites usually remains in subcutaneous regions of the body and do not migrate and lodge in the pulmonary artery or heart (Foissac et al, 2013).

Adult canine heartworms range from 10 to 30 cm in length, with males measuring between 10-15 cm and females measuring 25-30 cm, and both males and females are approximately 1 mm in diameter (American Heartworm Society, 2018). Their large size and location of attachment results in significant damage to the heart of the host. The adult parasites settle primarily in the right ventricle of the heart and the pulmonary artery running between the heart and lungs. As the number of adults in the heart increases, the burden on the heart becomes greater as the heartworm disease progresses. Affected dogs become increasingly intolerant to exercise due to decreased cardiac function, and cough frequently due to their enlarged heart pushing upwards against the

trachea (Miller and Gordon, 2009). The burden of the worms causes right-sided heart enlargement known clinically as cardiomegaly, which in turn causes the heart to function less efficiently. Eventually, congestive heart failure occurs when the heart is unable to pump out the volume fluid that it takes in, and this fluid accumulates in the lungs. Additionally, antigens secreted from the heartworms can cause severe regenerative anemia, leading to organ damage (Miller and Gordon, 2009). Both congestive heart failure and regenerative anemia play a role in the death of the host canine.

Consequences of infection by Dirofilaria immitis

Extensive research on the *D. immitis* lifecycle and transmission over the last 5-6 decades has resulted in a variety of prescription heartworm prophylactics available through veterinarians for pet owners to treat their dogs, cats, and ferrets in order to prevent a heartworm infestation. The most commonly prescribed prophylactic drugs are all classified as macrocyclic lactones: Moxidectin (injected or applied topically), Selamectin (applied topically), Ivermectin (given orally), and Milbemycin oxime (given orally). There are also therapies available for dogs with pre-existing heartworm infestations. The least involved option is the "slow-kill" treatment plan, where dogs take a monthly Ivermectin- based prophylactic medication for the duration of treatment (and continue to take afterwards for prevention of future infestations), as well as 30 days of twice-daily doses of the antibiotic Doxycycline or Minocycline, given every other month. Doxycycline and Minocycline kill *Wolbachia*, a rickettsial symbiotic bacterium of the canine heartworm (Papich, 2017). Eliminating *Wolbachia* can decrease pulmonary inflammation caused by *Wolbachia*, kill L4 stage canine heartworms which are in the process of migrating to the heart, and reduce the circulating number of microfilariae in the host canine (Papich, 2017). The slow-kill treatment plan does not kill adult heartworms but eliminates the risk of further infestation by administering a monthly prophylactic, and potentially weakens the existing adult heartworms which die on their own at the end of their life cycle. This method is not endorsed by the American Heartworm Society due to the risk of damage to the heart and other vital organs due to the continual presence

of adult heartworms throughout treatment (American Heartworm Society, 2018). The most extensive treatment plan is known as the "fast-kill" method and involves pre-treatment radiographs and blood chemistry panels, a monthly Ivermectin based prophylactic medication, and one month of twice-daily oral Doxycycline or Minocycline 30 days prior to injections (American Heartworm Society, 2018). An injection of an adulticide drug called Melarsomine is given intramuscularly to the canine with the purpose of killing adult heartworms, and Prednisone is prescribed as an anti-inflammatory and immune suppressant to prevent reaction to dying adult heartworms. Thirty days later, the dog receives two more injections of Melarsomine given 24 hours apart and is prescribed another 30 days of Prednisone (American Heartworm Society, 2018). After six months, the dog is retested with an antigen ELISA test, and in most cases, is heartworm-negative (American Heartworm Society, 2018).

Although prophylactic medications are available in multiple forms from various sources including veterinarians and pet pharmacies, several factors including lack of owner education, owner apathy, owner's financial restraints, and pet homelessness attribute to numerous reported cases of Canine Heartworm Disease in domestic dogs in the Southeast (American Heartworm Society, 2018). Additionally, the emergence of a *D. immitis* strain resistant to macrocyclic lactone heartworm preventatives in the Lower Mississippi Delta region of the United States, recorded since 2005 (Pulaski et al, 2014) indicates the parasite's ability to evolve and a potential need to adapt additional approaches to heartworm prevention in pets such as vector control. This study aims to provide information on the role that wild canids play in the *D. immitis* lifecycle as reservoir hosts. Coyotes in the Southeastern region of the United States are proven hosts of *D. immitis*, with recent investigations finding 51% prevalence in specimens collected at B.F. Grant Wildlife Management Area and Cedar Creek Wildlife Management Area in Georgia (Gates et al, 2014), 37% prevalence in specimens collected throughout 28 counties in Florida (Aher et al, 2016), 29% prevalence in specimens examined from the Savannah River Site in South Carolina (Miller et al, 2009), and 47% prevalence in specimens from Fort Bragg, North Carolina (Chitwood et al, 2015).

Role of Canis latrans in Dirofilaria immitis transmission

Due to the nature of *D. immitis* transmission from infected dog to mosquito to another dog, it is logical to infer that canines which are not consistently receiving prophylactic anti-filarial medication are at risk of becoming infected with the parasite if they live in close proximity to an infected host, as they are exposed to the same population of mosquitoes. With residential coyotes roaming areas of about 10 square miles and transient coyotes travelling 50-100 miles at a time, a coyote playing host to *D. immitis* has the potential to introduce microfilariae into mosquito populations in both rural and suburban to urban areas as it migrates. Additionally, the coyote can be used as a model to evaluate the potential risk for infection by *D. immitis* in dogs not receiving a prophylactic anti-filarial. Unlike the portion of the domestic dog population which receive prophylactic treatment from their owners, coyotes are at a higher risk of contracting canine heartworm disease. Their lack of prophylactic treatment, continuous exposure to mosquitoes, and genetic similarities to domesticated dogs make them an ideal study species for assessing the risk of infection by *D. immitis*.

MATERIALS AND METHODS

Collection of Organs

Coyotes (collected as described in Ch. 1) were positioned in dorsal recumbency on a dissection table or on the tailgate of a truck in the case of field dissections. A piece of rope was attached to each limb and tied a stationary object to stabilize the coyote throughout dissection, similar to how canine patients are positioned and secured to the operating table during surgery (Figure 2.2). For all specimens dissected, the post-mortem interval was too advanced to allow for collection of blood via venipuncture. Therefore, all blood samples collected were pulled from anticoagulated blood and pleural fluid present in the thoracic cavity and deposited into a labeled blood tube containing a lithium-heparin additive for later testing.

Figure 2.2: Coyote positioned on a table for dissection. Thoracic limbs were typically extended further cranially; however, this specimen was still in the process of thawing.

Dissection of each coyote was performed using sharp/blunt straight surgical scissors and a lockback-skinner knife, which was disinfected with an aqueous 10% bleach solution and manual scrubbing with a brush and paper towels between each specimen. A deep incision was made along

the ventral midline through the entirety of the sternum extending posteriorly to the diaphragm to access the heart. The pericardial sac was opened, and incisions were made as distally as possible on the pulmonary artery, pulmonary vein, inferior (caudal) and superior (cranial) vena cava, and aorta in order to remove the heart from the chest cavity. The heart was then placed in a Ziploc bag with a solution of 70% Ethanol to preserve for further evaluation.

Organs removed from each coyote were preserved in 70% Ethanol, transported to the laboratory, and stored in the refrigerator at 4°C for later dissection under a fume hood. The heart was examined for heartworms, *Dirofilaria immitis*, by carefully using a scalpel equipped with a #10 blade and/ or small dissection scissors to open arteries and veins attached to the heart, followed by dissection of both atria and ventricles, to remove all worms although the majority were located in the pulmonary artery and right ventricle. Adult heartworms were carefully removed from the heart and rinsed with saline to remove coagulated blood, then placed in a solution of 70% Ethanol and 5% Glycerin for preservation. They were then quantified and identified as male or female based on sexually dimorphic characteristics. Additionally, an Idexx SNAP 4Dx test was used to analyze blood samples collected- while this was primarily used to assess exposure to tick-borne pathogenic bacteria (as described in greater detail in Chapter 4), the device also measured *D. immitis* antigen in the bloodstream. A positive or negative result was recorded for *D. immitis* antigen.

Statistical Analysis

A total of 38 coyotes (19 Males and 19 Females) were analyzed for infection by heartworm disease through manual dissection. A 2-sample t-test was run to determine differences in both infection rate and number of adult *D. immitis* present in relation to sex of coyotes. Analysis of Variance (ANOVA) tests were run separately to determine the relationship between Geographic regions in Georgia, DNR management regions in Georgia, and approximate age on the number of adult *D. immitis* present in coyotes.

Evaluation of Mosquito Population for larval Dirofilaria immitis

In order to assess the burden of *D. immitis* in mosquito populations present in the coyote's habitat, CDC Gravid Traps (model 1712 – J.W. Hock Company), (Figure. 2.3) were used to collect mosquitoes. Gravid trapping was conducted opportunistically between July 2018 and May 2019. Gravid female mosquitoes were attracted to the traps by using a mixture of water, chicken manure, and hay/grass clippings (colloquially known as stink bait) which imitates the organically polluted, stagnant bodies of water where female mosquitoes oviposit. Once female mosquitoes flew down to land and oviposit on the bait, they were propelled upwards through a tube into a net by a battery powered updraft fan. Circular metal pizza cooking sheets were balanced on top of the nets to help protect the contents of the trap in the event of rainfall during trapping. Locations were determined for trapping by assessing GPS coordinates or approximate locations of kills provided by coyote hunters and assessing the surrounding areas with Google Maps Satellite technology and physically to locate a level area to set the trap as close to a source of water and vegetation as possible (Figure 2.3). Traps were set at dusk and collected at sunrise the following morning. In ideal situations, the nets were transported to the lab in a cooler lined with moist paper towels and frozen for 5-10 minutes to immobilize and kill insects present in the net, however in some situations traveling to multiple trapping sites throughout Georgia prevented immediate return to the lab. In these scenarios, the mosquitoes were frozen to immobilize and separate from other insects in the net, transferred to a labeled polystyrene petri dish, placed in a sealable Tupperware container lined with moist paper towels, and stored in a refrigerator and/or iced cooler.

Figure 2.3: Gravid traps were placed as close to water, vegetation, or both when possible.

In the lab, mosquitoes were counted, and identified to species and sex using species descriptions from "Mosquitoes of the Southeastern United States" (Burkett-Cadena, 2013). The specimens were then manually dissected on a glass microscope slide using 2 nematode picks (a small dissecting probe with a thin, braided metallic needle) and a solution of physiological saline (0.8% NaCl). While looking through a dissecting microscope, 2-3 drops of 0.8% NaCl was dropped using an 8mL transfer pipet directly on top of the mosquito, which was then held down at the junction of the thorax and abdomen with one pick, while the other pick was used to apply gentle pressure slightly anterior to the tip of the abdomen. The picks were then pulled slowly in opposite directions to separate the tip of the abdomen from the rest of the body, with the intent to pull the mosquito's digestive tract out of the abdomen and maintain the integrity of structures like the Malpighian Tubules. The thorax and head were then gently teased apart with needles to separate the darker exoskeleton from internal structures, and organs were spread out into a thin layer on the slide for examination. A wet-mount was created by placing a coverslip over the mosquito and applying gentle pressure, and the slide was transferred to a compound microscope and examined

for the presence of larval stages of *D. immitis*. Scanning for structures of importance on the slide was performed at 100X total magnification, and 400X total magnification was used for more detailed observation. The presence or absence of suspected larval *D. immitis* was recorded, and photographs were taken through the lens of the microscope for any specimens which appeared to be positive. Additionally, any specimens that appeared positive were transferred to individual microcentrifuge tubes containing 90% ethanol to be tested for the presence of *D. immitis* DNA via PCR in future experiments.

RESULTS AND DISCUSSION

Results for infection of Canis latrans by Dirofilaria immitis

Coyotes originating from 6 of Georgia's DNR management regions and 4 of its geographic regions were examined for the presence of adult *Dirofilaria immitis* (Tables 2.1 & 2.2). Of the 38 coyotes collected between December 2017 and March 2019, 21 had adult *D. immitis* physically present in the heart and lung tissue examined, with the overall prevalence of *D. immitis* in the sample population being 55.26%. There was no significant trend in infection by *D. immitis* between sexes (n=38, df=1, p=0.7523): 10 out of 19 males (52.63%) and 11 of 19 females (57.9%) tested positive. The average number of adult *D. immitis* per infected coyote was 17.1 (n=21, std. dev. 18.6), with the heaviest burden being 63 adult worms (Table 2.3, Table 2.4, Figure 2.4, Figure 2.5). Number of adult heartworms found per coyote did not vary significantly among the 4 different age groups (n=38, df=3, p=0.4511), and did not vary significantly among sex (n=38, df=1, p= 0.3). ANOVA testing showed no correlation between the approximate age of coyotes and the burden of *D*. immitis (n=38, df=3, Prob>F=0.4511). The ratio of adult female to male heartworms was 1.06:1. Prevalence (frequency) of heartworm infection in coyotes sampled was not dependent on approximate age of the coyote (n=38, df=3, Pearson $P>X^2$=0.3362) (Figure 2.6), DNR Management region (n=38, df=5, Pearson $P>X^2$= 0.959) (Figure 2.7), or geographic region (Pearson $P>X^2$= 0.367, df=3, n=38) (Figure 2.8) . The SNAP 4Dx ELISA *D. immitis* antigen test had a 96.55% sensitivity and specificity rate for correctly diagnosing infection by *D. immitis*.

Table 2.1: Number of coyotes testing positive for adult *D. immitis* by DNR Management region.

DNR Management Region	**Coyotes Positive for *D. immitis***	**Total Coyotes Tested**
Northeast	4	6
Northwest	3	6
East Central	2	4
West Central	3	7
South Central	5	8
Southwest	4	7

Table 2.2: Number of coyotes testing positive for adult *D. immitis* by geographic region.

Geographic Region	**Coyotes Positive for *D. immitis***	**Total Coyotes Tested**
Ridge and Valley	0	2
Piedmont	11	19
Upper Coastal Plains	7	13
Lower Coastal Plains	3	4

Table 2.3: Number of *D. immitis* present per coyote by DNR Management region.

DNR Management Region	Number of *D. immitis* present	Total Positive/ Total Tested
Northeast	0	4/6
	0	
	4	
	4	
	40	
	59	
Northwest	0	3/6
	0	
	0	
	2	
	14	
	17	
East Central	0	2/4
	0	
	1	
	3	
West Central	0	3/7
	0	
	0	
	0	
	3	
	4	
	22	
South Central	0	5/8
	0	
	0	
	2	
	12	
	12	
	30	
	38	
Southwest	0	4/7
	0	
	0	
	3	
	11	
	15	
	63	

Table 2.4: Number of *D. immitis* present in coyotes by geographic region.

Geographic Region	Number of *D. immitis* present	Total Positive/ Total Tested
Ridge and Valley	0	0/2
	0	
Piedmont	0	11/19
	0	
	0	
	0	
	0	
	0	
	0	
	0	
	1	
	2	
	3	
	3	
	4	
	4	
	14	
	17	
	22	
	40	
	59	
Upper Coastal Plains	0	7/13
	0	
	0	
	0	
	0	
	0	
	3	
	4	
	11	
	12	
	15	
	30	
	63	
Lower Coastal Plains	0	3/4
	2	
	12	
	38	

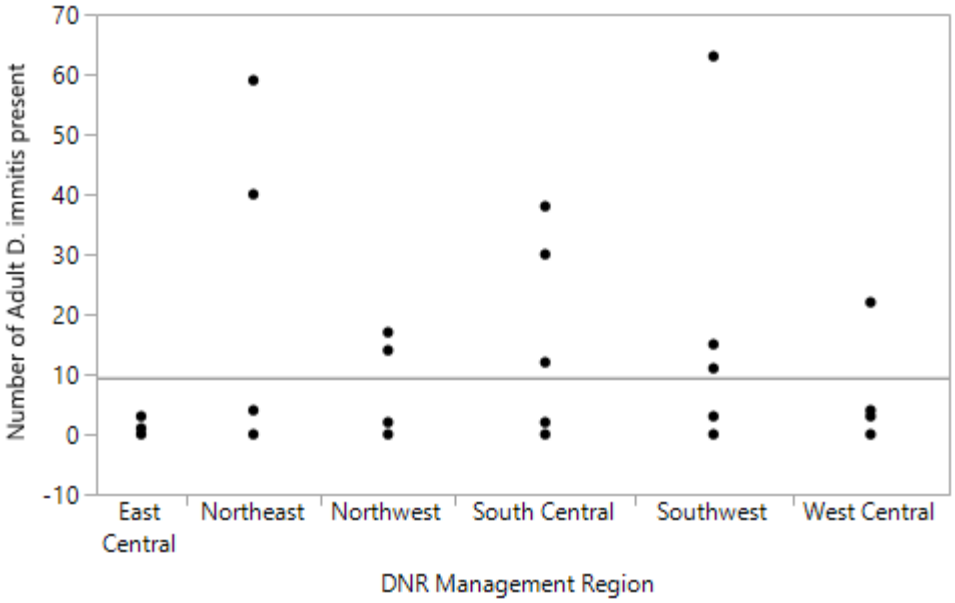

Figure 2.4: Number of adult *D. immitis* present per coyote based on DNR management region.

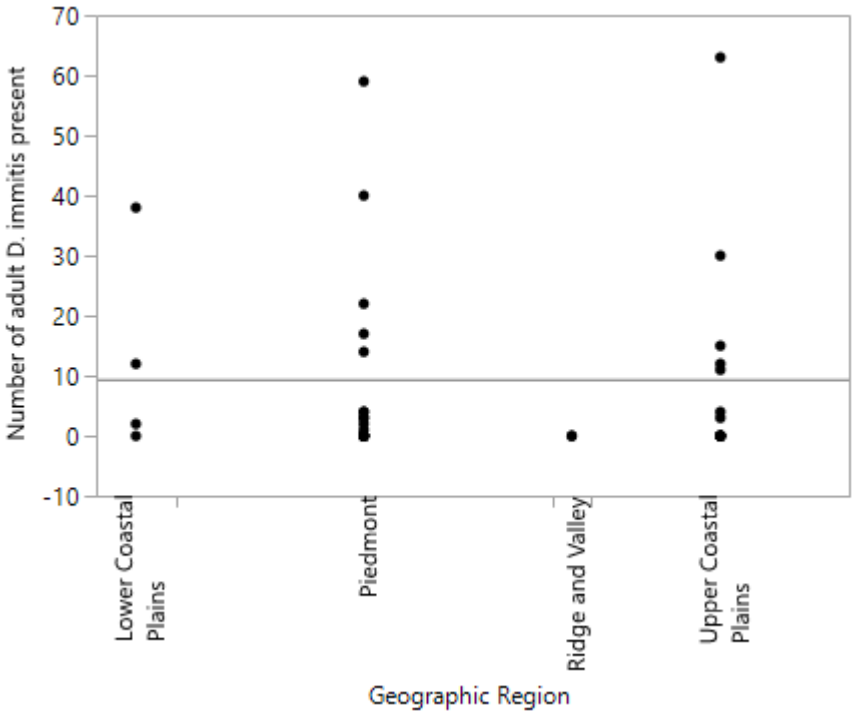

Figure 2.5: Number of adult *D. immitis* present per coyote based on geographic region.

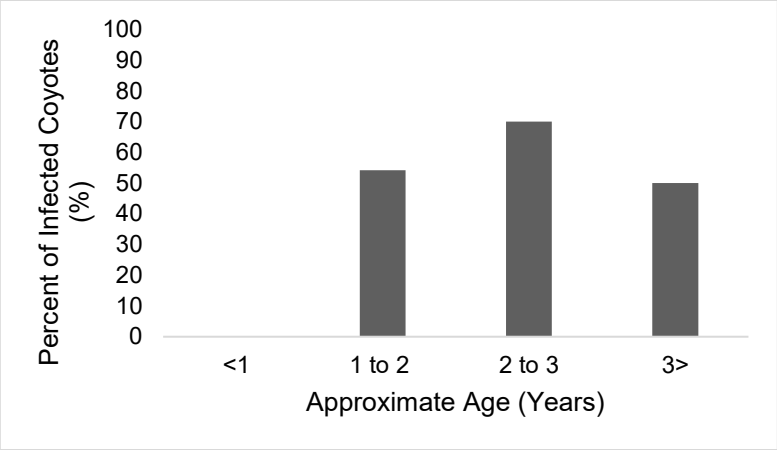

Figure 2.6: Prevalence of *D. immitis* in coyotes by approximate age range.

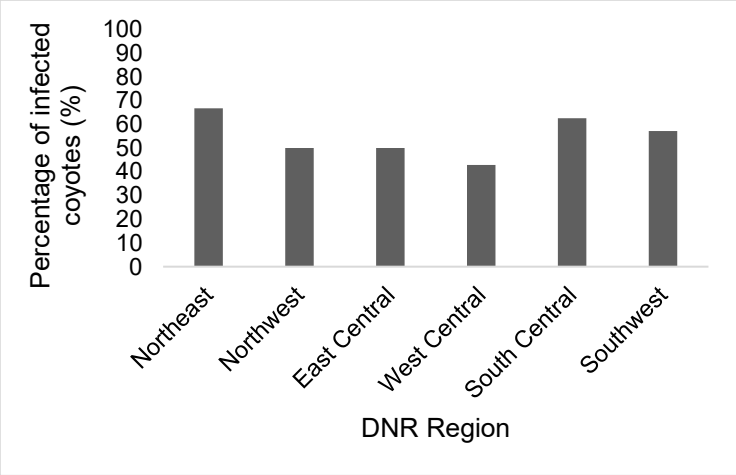

Figure 2.7: Prevalence of *D. immitis* infection in coyotes by DNR management region.

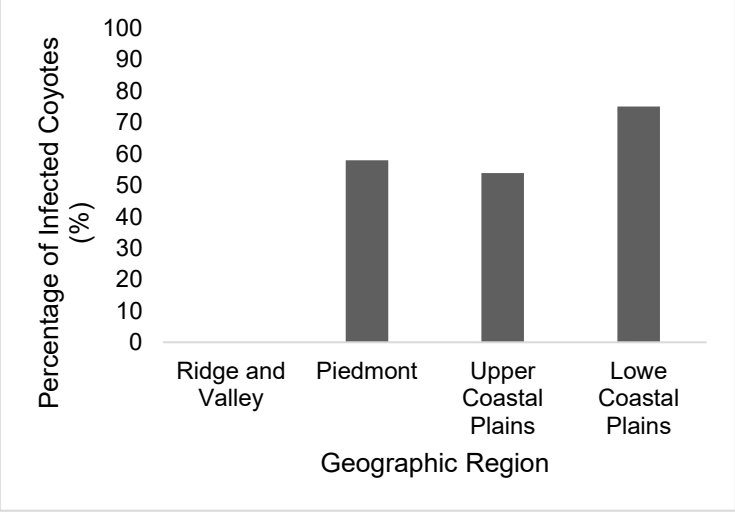

Figure 2.8: Prevalence of *D. immitis* infection in coyotes by geographic region.

Results for infection of mosquito vectors with larval Dirofilaria immitis

Gravid trapping on 12 separate occasions yielded a total of 148 testable mosquitoes (Table 2.5), of which 129 were gravid, 3 were recently bloodfed, and 16 were unfed. Nine species of mosquitoes were collected, with *Culex quinquefasciatus* and *Culex restuans* occurring most frequently (Table 2.5; Figure 2.9). Seven of the nine species collected are known vectors of *D. immitis* (Table 2.5). Only 3 of the 148 mosquitoes collected were suspected to be positive for larval *D. immitis* (Table 2.9).

Table 2.5: Results for gravid trapping of mosquitoes in areas proximate to coyote location.

County	Geographic Region	DNR Management Region	Dates Trapped	Species (Number)	Heartworm Vector
Terrell	Upper Coastal Plains	Southwest	July 03 2018-July 04 2018	*Culex tarsalis* (1)	Yes
Twiggs	Upper Coastal Plains	West Central	July 03 2018-July 04 2018	*Culex quinquefasciatus* (3)	Yes
Jones	Piedmont	West Central	July 03 2018-July 04 2018	N/A (0)	N/A
Terrell	Upper Coastal Plains	Southwest	July 18 2018-July 19 2018	*Aedes albopictus* (2)	Yes
				Aedes canadensis (1)	Yes
				Culex quinquefasciatus (1)	Yes
Sumter	Upper Coastal Plains	Southwest	July 20 2018-July 21 2018	*Aedes albopictus*(4)	Yes
				Aedes canadensis (1)	Yes
				Culex quinquefasciatus (1)	Yes
Dooly	Upper Coastal Plains	Southwest	July 22 2018-July 23 2018	*Culex quinquefasciatus* (1)	Yes
Dooly	Upper Coastal Plains	Southwest	August 17 2018-August 18 2018	*Culex quinquefasciatus* (18)	Yes
Sumter	Upper Coastal Plains	Southwest	August 17 2018-August 18 2018	*Aedes albopictus* (1)	Yes
				Culex quinquefasciatus (5)	
Terrell	Upper Coastal Plains	Southwest	August 18 2018-August 19 2018	N/A (0)	N/A
Sumter	Upper Coastal Plains	Southwest	September 04 2018-September 05 2018	*Aedes albopictus* (1)	Yes
				Culex quinquefasciatus (4)	Yes
				Culex nigripalpus (2)	Yes
Dooly	Upper Coastal Plains	Southwest	September 14 2018-September 15 2018	*Culex quinquefasciatus* (38)	Yes
Twiggs	Upper Coastal Plains	West Central	September 14 2018-September 15 2018	*Culex quinquefasciatus* (1)	Yes
Jones	Piedmont	West Central	September 14 2018-September 15 2018	*Culex quinquefasciatus* (1)	Yes

Wheeler	Upper Coastal Plains	South Central	April 05 2019-	*Culex restuans* (17)	Yes
			April 07 2019	*Culex salinarius* (1)	Yes
				Culex quinquefasciatus (1)	Yes
Toombs	Lower Coastal Plains	South Central	April 05 2019-	*Culex restuans* (2)	Yes
			April 06 2019	*Culex salinarius* (2)	Yes
Wheeler	Upper Coastal Plains	South Central	April 12 2019-	*Culex quinquefasciatus* (6)	Yes
			April 13 2019	*Orthopodomyia signifera* (1)	No
Toombs	Lower Coastal Plains	South Central	April 12 2019-	*Culex restuans* (7)	Yes
			April 13 2019	*Culex salinarius* (1)	Yes
Franklin	Piedmont	Northeast	May 22 2019-	*Culex restuans* (6)	Yes
			May 23 2019		
Wilkes	Piedmont	East Central	May 22 2019-	*Culex restuans* (6)	Yes
			May 23 2019	*Culiseta melanura* (1)	No
				Orthopodomyia signifera (1)	No
Gordon	Ridge and Valley	Northwest	May 27 2019-	*Aedes canadensis* (1)	Yes
			May 28 2019		
Bartow	Ridge and Valley	Northwest	May 27 2019-	*Culex restuans* (1)	Yes
			May 28 2019		
Gwinnett	Piedmont	Northeast	May 27 2019-	N/A (0)	N/A
			May 28 2019		
Troup	Piedmont	West Central	May 27 2019-	*Culex restuans* (4)	Yes
			May 28 2019	*Orthopodomyia signifera* (1)	No
Pickens	Piedmont	Northwest	May 27 2019- May 28 2019	*Aedes albopictus* (1)	Yes
				Culex restuans (2)	Yes

Table 2.6: Total numbers of mosquitoes collected in gravid traps from July 2018 to May 2019.

Mosquito Species	**Number collected**
Aedes albopictus	9
Aedes canadensis	3
Culex nigripalpus	2
Culex quinquefasciatus	80
Culex restuans	45
Culex salinarius	4
Culex tarsalis	1
Culiseta melanura	1
Orthopodomyia signifera	3

Table 2.7: Mosquito species collected in gravid traps by geographic region.

Geographic Region	Species Collected	Quantity Collected
Ridge and Valley	*Aedes canadensis*	1
	Culex restuans	1
Piedmont	*Aedes albopictus*	1
	Culex quinquefasciatus	1
	Culex restuans	18
	Culiseta melanura	1
	Orthopodomyia signifera	2
Upper Coastal Plains	*Aedes albopictus*	8
	Aedes canadensis	2
	Culex nigripalpus	2
	Culex quinquefasciatus	79
	Culex restuans	17
	Culex salinarius	1
	Culex tarsalis	1
	Orthopodomyia signifera	1
Lower Coastal Plains	*Culex restuans*	9
	Culex salinarius	3

Table 2.8: Mosquito species collected in gravid traps by DNR management region.

DNR Management Region	Species Collected	Quantity Collected
Northeast	*Culex restuans*	6
Northwest	*Aedes albopictus*	1
	Aedes canadensis	1
	Culex restuans	3
East Central	*Culex restuans*	6
	Culiseta melanura	1
	Orthopodomyia signifera	1
West Central	*Culex quinquefasciatus*	5
	Culex restuans	4
	Orthopodomyia signifera	1
South Central	*Culex quinquefasciatus*	7
	Culex restuans	26
	Culex salinarius	4
	Orthopodomyia signifera	1
Southwest	*Aedes albopictus*	8
	Aedes canadensis	2
	Culex nigripalpus	2
	Culex quinquefasciatus	68
	Culex tarsalis	1

Table 2.9: Location, date collected, and species of mosquitoes suspected to be positive for *Dirofilaria immitis* larvae.

County	Geographic Region	DNR Region	Date Trapped	Species	Suspected Positive Results
Terrell	Upper Coastal Plains	Southwest	July 18 2018-July 19 2018	*Aedes albopictus*	1
				Aedes canadensis	1
Troup	Piedmont	West Central	May 27 2019-May 28 2019	*Culex restuans*	1

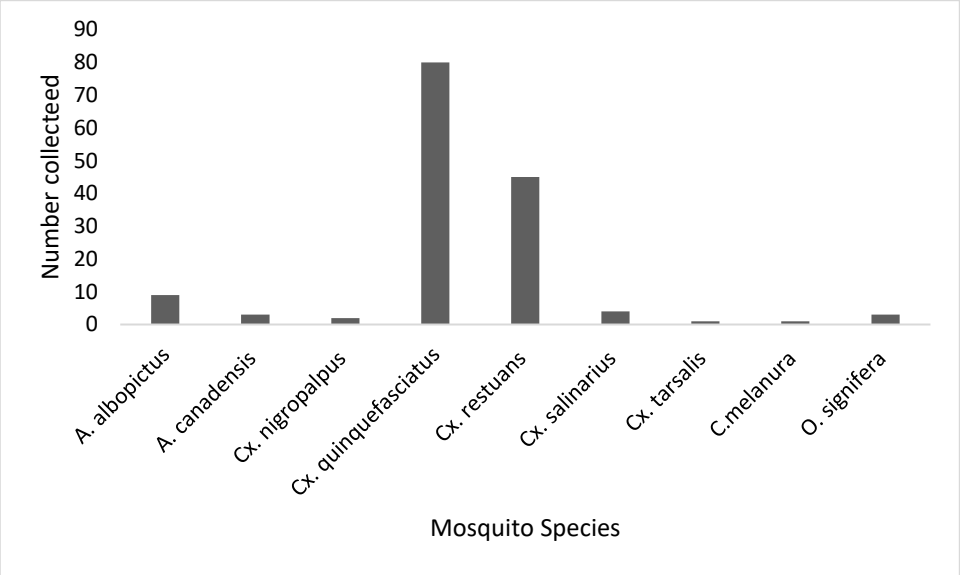

Figure 2.9: Distribution of mosquito species collected with gravid traps from July 2018 to May 2019.

Prevalence of Dirofilaria immitis in Canis latrans

The prevalence of *Dirofilaria immitis* infection in this study's sample population (55.26%) was consistent with, and slightly greater than results from recent studies from the Southeastern United States, with 51% in central Georgia (Gates et al, 2014), 29% in South Carolina (Miller et al, 2009), 37% in Florida (Aher et al, 2016), and 47% in North Carolina (Chitwood et al, 2015). Lack of correlation between approximate age and number of *D. immitis* found per specimen is not unexpected or unusual, as the majority of the samples were classified as young to middle-aged adults (n=36) within a 3-year range. The two juvenile specimen estimated to be less than a year of age were both negative for heartworms, which was expected as *D. immitis* larvae take 6 months to

mature to adulthood after infection occurs (Venco et al, 2015). If adult heartworms had been present in the juvenile coyotes, the burden would likely have been very low. Similarly, if a greater quantity of middle-aged and older adults had been included, a more noticeable correlation between age and burden of infection may have been observed, as older coyotes have had more exposure to mosquitoes and are therefore more likely to have higher burdens of *D. immitis,* which has been historically supported throughout past decades and was found in a recent study of coyotes in North Carolina (Chitwood et al, 2015). Lack of correlation between approximate weight and number of *D. immitis* is consistent with results from Florida (Aher et al, 2016) and Illinois (Nelson et al, 2003). The 1.06:1 Female: Male ratio in the sample population contained a similar frequency of female heartworms to the 1.14:1 ratio found in a study of *D. immitis* burden in Florida's coyote population (Aher et al, 2016). Prevalence of infection by *D. immitis* in the sample population varied slightly among DNR management regions with the lowest rate being 50% in the Northwest and East Central regions to the highest rate of 66% in the Northeast region. Ranking by geographic region was more variable, with 0% prevalence in the sample population from the Ridge and Valley Region (n=2), and 75% in the Lower Coastal Plains region (n=4); however, the extremely low sample size in these 2 regions cause a single positive or negative result to weigh too heavily to draw any major conclusions from these results.

The average number of adult *D. immitis* among the 21 infected coyotes was 17.1 heartworms; 9 coyotes had infections with less than 10 adult heartworms, 6 with 10-20 adult heartworms, 4 with 20-40 adult heartworms, and two specimens had a large burden (59 & 63 heartworms retrieved). While adult heartworms typically lodge in the right atrium, right ventricle and pulmonary artery, the two coyotes with heavy burdens of adult heartworms had worms present in all 4 chambers of the heart as well as the pulmonary artery and inferior vena cava (Figure 2.10). This observed location of the heartworms in the left chambers of the heart is unusual and not supported by clinical reports and studies on heartworm disease, and is likely due to the settling of worms and bodily fluids post-mortem, accompanied by movement and turning of the carcass during transport and

dissection. However, the presence of *D. immitis* in the inferior vena cava is well documented in canines in cases of caval syndrome due to heavy heartworm burdens (Chikweto et al, 2014), and has also been observed in coyotes (Miller et al, 2007). The reported observance of *D. immitis* present in the inferior vena cava occurred in a coyote from the Savannah River Site study area in South Carolina whose cause of death was a ruptured aortic aneurysm of unknown etiology, theorized to potentially be secondary to presence of *D. immitis* in the vena cava (Miller et al, 2007). While the size of a specimen and its organs will naturally vary amongst organisms, hearts examined which contained a heavy burden of adult heartworms were notably larger than those with no infection or a light infection (Figure 2.11), indicative of cardiomegaly associated with heavy infection with *D. immitis*.

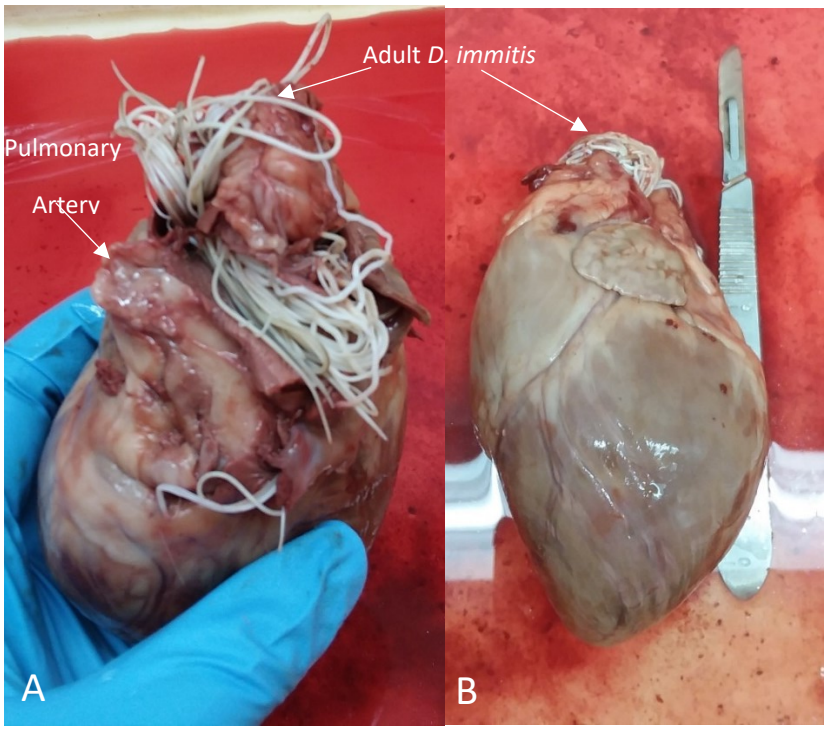

Figure 2.10: Coyote heart with a burden of 63 adult worms retrieved, shown from right side of heart (A), shown next to scalpel for scale (B).

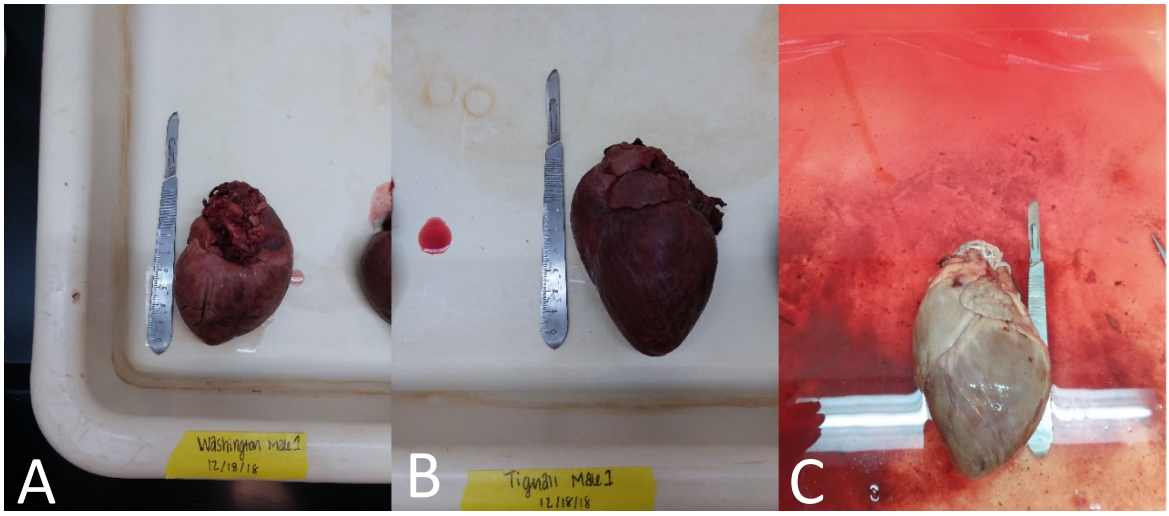

Figure 2.11: Comparison of a heart free of heartworms (A), with a light burden of heartworms (B), and a heavy burden of heartworms (C) with scalpel included for scale.

The SNAP 4Dx ELISA antigen test for *D. immitis* had a 96.66% sensitivity and specificity rate for correctly diagnosing heartworm infection- 29 of 30 tests matched the results from physical dissection. The one inconsistent result was a light positive indication dot on the SNAP 4dx test from a 1 to 2 year-old male from Wilkes County, GA, which had no adult *D. immitis* found on dissection. This could potentially be an error in sampling by failure to remove any adult heartworms present from the coyote. Although every effort was made to remove the heart and its major attached vessels where the heartworms reside, this was not possible in every dissection due to the difficulty of one individual having to manually hold the chest cavity open while isolating and detaching major vessels from the body. While the heart and lungs were only able to be removed from the thoracic cavity still attached in a few instances (Figure 2.12), this method is more conducive to collecting higher yields of adult heartworms without damaging or cutting any in half while removing the organs from the body. Furthermore, the intensity of the indication dot varies among specimens and becomes darker in shade as the burden of heartworms increases, an infection with a light burden of heartworms yields a light blue indication dot while a higher burden of worms will yield a darker blue indication dot (Figure 2.13). The pale color of the dot on the SNAP test

indicates an infection with a light burden of worms, perhaps only 1-2 total adult worms present, increasing the likelihood that the dissector could miss a physical infection of *D. immitis*.

Figure 2.12: Heart and lungs still connected after removal from coyote.

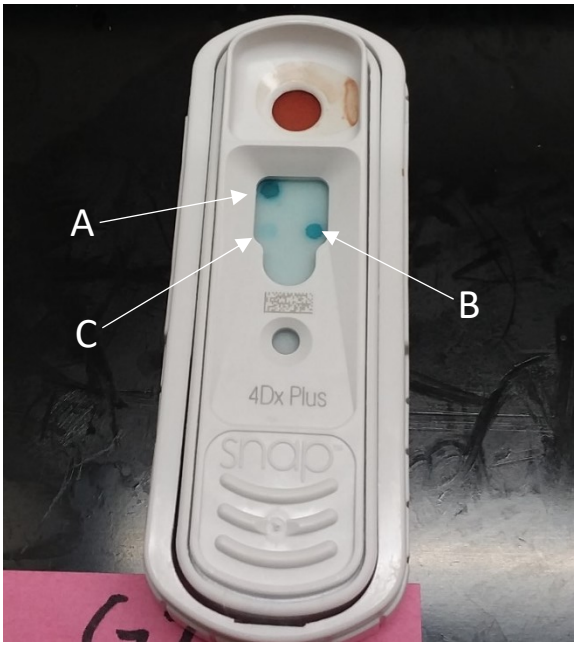

Figure 2.13: A SNAP 4Dx device with results varying in shade intensity: the dark blue dot is the test's positive control (A), medium blue dot (B) indicates presence of a moderate amount of *D. immitis* antigen present (12 adults), and the light blue dot is indicative of a small (light) amount of antibodies against *Ehrlichia* spp. bacteria (C) present in the blood sample tested.

Analysis of larval Dirofilaria immitis in mosquito populations

Trapping for mosquitoes occurred overnight on 12 separate occasions, and a total of 17 locations were sampled, all of which were in proximate locations to where the coyotes analyzed in this study were collected. Due to hematophagous behavior displayed only by female mosquitoes, gravid trapping to select for females was the chosen method of collection. While other devices such as CDC light traps are known to collect a larger variety of species, gravid traps are known to collect higher yields of mosquitoes, and collected 6.17X more than CDC light traps in a surveillance study in Texas, which also revealed gravid traps to have slightly higher success rates in selecting for female mosquitoes than CDC traps (76.7% vs 73.9%) (White et al, 2009). In total, 167 mosquitoes were collected, of which 148 were females able to be identified and dissected, 6 were male, and 13 discarded as they were too damaged to identify, i.e. missing entire sections of the body or smashed. The gravid traps and bait used in this study yielded 96.1% female mosquitoes and 3.9% male mosquitoes.

When a water source was proximate to the coyote's coordinates of origination, gravid traps were placed as close to the water as possible, whether it be a pond, swamp, stagnant creek, or stagnant area of water in a roadside drainage ditch, (Figure 2.3). The placement of traps near water sources was chosen based on gravid female mosquitoes' biological need to locate stagnant bodies of water for oviposition (Mullen and Durden Ch. 14, 2009), with the hope that the trapping bait may be more attractive than the nearby water source. The possibility that placing traps proximate to water sources may not be advantageous due to the much larger surface area of readily available stagnant water for oviposition was also considered in cases when the available property for trapping did not contain a water source. In these instances, traps were placed near areas of vegetation and under tree cover when possible to help shelter traps from potential rainfall; however, these locations were minimally successful. Out of 5 sampling attempts in 3 different counties, the traps were empty on 2 occasions, one mosquito was found per trap on 2 occasions,

and 3 per trap at one location. There was also one instance of a trap set by a water source (in a swamp) which yielded no mosquitoes.

The number of mosquitoes collected by gravid trapping was unfortunately much lower than anticipated. In one instance, unpredicted overnight showers caused the level of bait to rise in the tub which the fan-containing tube is propped over, resulting in the bottom of the tube where mosquitoes enter being completely submerged and thus pulling in no mosquitoes. Various other factors are likely to have played a role including lack of recent rainfall and pesticide application, as the majority of trapping locations were located on land used for agricultural purposes. Efficacy of the bait used may also vary due to the ratio of water, grass clippings, and chicken manure is constantly changing as it is depleted by researchers, refreshed with manure, clippings, water, and rainfall. Additionally, the results of mosquitoes collected using the same traps and bait as this study yield variable results on a week-week basis at three locations in Bulloch County Georgia, with some collections resulting in few to no specimens while one trap yielded over 600 specimens in one night in June 2017 (Personal communication and experience). Due to the amount of travel required to reach most trapping locations from the research institution and the separate trips required to set and retrieve traps, trapping was conducted opportunistically, with the Southwest/ South Central/ West Central DNR regions sampled more frequently due to the investigator's access to overnight lodging in a location central to 5 trapping sites.

Nine species of mosquitoes were collected in total, seven of which are known vectors of *Dirofilaria immitis*: *Aedes albopictus, Aedes canadensis, Culex nigripalpus, Culex quinquefasciatus, Culex restuans, Culex salinarius, and Culex tarsalis* (Figure 2.14). *Aedes albopictus, Aedes canadensis*, and *Culex quinquefasciatus* (all collected in this study) among several other species present in Georgia have been identified as species whose presence is a significant risk factor for heartworm infection in the United States (Wang et al, 2014). A study using PCR primers to detect *D. immitis* in mosquito populations collected by gravid and CDC light traps in Georgia found *Aedes albopictus* to have the highest infection rate of species collected

(Licitra et al, 2010). Out of 148 total dissections, 3 individual mosquitos belonging to the species *Aedes albopictus, Aedes canadensis,* and *Culex restuans* appeared to be infected with larval *D. immitis* upon dissection (Figure 2.15). While these specimens appeared to contain *D. immitis* larvae, it is impossible to be certain of an infection without genetic analysis of samples by PCR. It is possible that larval *D. immitis* were overlooked in mosquitoes during dissection due to the difficulty of locating and distinguishing early larval stages inside the mosquito (Saurman and Nayer, 1983). Additionally, the infection rate with *D. immitis* in Georgia's mosquito population tested by the more accurate PCR analysis method was still low,with a total of 15 pooled groups of mosquitoes testing positive out of a total 1,401 mosquitoes (Licitra et al, 2010). With those factors taken into account, the small amount of suspected positives in this study is not significantly lower than one would expect.

Figure 2.14: Various mosquito species trapped and photographed before dissection.


Figure 2.15: Image of suspected melanized *D. immitis* larvae found during dissection of *Culex restuans* mosquito from Troup Co., GA (Piedmont/ West Central Region).

Implications of Canis latrans as a wildlife reservoir host for Dirofilaria immitis

This study provides a greater understanding of *Dirofilaria immitis* prevalence and burden covering a larger area of the state of Georgia than previous works. While sample size varied slightly amongst the different regions analyzed, the results were consistent amongst regions, indicating no unique patterns of infection prevalence based on location of origin. Considering the statewide presence of the important vector species *Aedes canadensis, Aedes trivittatus, Anopheles punctipennis, Anopheles quadrimaculatus*, and *Culex quinquefasciatus* (with the exception of *Aedes trivittatus* which is absent from the southern-most counties of the state), the even distribution of heartworm prevalence amongst regions of Georgia is logical (Wang et al, 2014). The ability to link incidence of heartworm disease reported in domestic canines to infection in coyotes in the vicinity would be an interesting and useful future direction for research and help to strengthen the understanding of coyotes as a risk factor for *D. immitis* infection in domestic canines. Additionally, genetic analysis to determine haplotypes of the *D. immitis* collected in coyotes in comparison to *D. immitis* found in domestic canines will help to better assess whether the haplotypes of *D. immitis* in coyotes and domestic canines vary (Aher et al, 2016). The emergence of *D. immitis* resistant to prophylactic macrocyclic lactones in the Southeastern United States within the last decade indicates the need for a more in depth understanding of distribution and variation amongst heartworms in their canid hosts (Pulaski et al, 2014). In conclusion, the 55.26% prevalence of *D. immitis* infection found in the sample population illustrates that the parasite is abundantly present in Georgia's coyotes. High prevalence of heartworm among coyotes combined with the presence of seven species of mosquitoes which act as vectors for *D. immitis* indicates a significant risk for heartworm infection in domestic canines which do not receive prophylactic treatment.

CHAPTER 3

INTESTINAL PARASITES

Mutual Enteric Parasites of Canis latrans, domestic canines, and humans.

In addition to introducing filarial parasites to the mosquito population, coyotes also have the potential to expose domestic pets and humans to enteric (intestinal) parasites by defecating in yards and residential areas. Coyotes play host to a variety of intestinal parasites which can also infect domestic pets such as tapeworms, roundworms, hookworms, and whipworms (Redman et al, 2016).

The most common species of hookworms (Nematodes belonging to the Family Ancylostomidae) found in coyotes were *Ancylostoma caninum*, the Canine Hookworm, a small species ranging 1 to 2 cm in length, and *Uncinaria stenocephala,* the Northern Canine Hookworm, a slightly larger species ranging from 3 to 12 cm in length (Seguel and Gottdenker, 2017). Infection with *Ancylostoma* spp. hookworms is extremely common in domestic canines in the United States, and most areas of the world. While the incidence in humans is not extremely common, *Ancylostoma caninum* is capable of infecting and causing pathogenicity in humans as well as canids (Provic and Croese, 1990). When a host is infected with hookworms, adults live and reproduce in the intestine, producing ova which exit the animal's body in feces. The ova hatch into L1 larvae which are non-infective and known as rhabiditiform larvae (Anderson, 2000). After approximately 48 hours, the L1 larvae molt to the L2 stage, and after 96-120 hours, the L2 molt to an L3 larvae. The L3 larvae are called filariform, and in cases of *Ancylostoma* spp., are capable of penetrating the skin of a host and migrating though body tissues to make their way to the host, while L3 larvae of *Uncinaria* spp. are ingested by their host (Anderson, 2000). Heavy infections of *Ancylostoma caninum* have proven lethal in coyote pups at densities of 300 larvae/ kg (Radomsky, 1989), and are linked to anemia, retarded growth, and tissue damage in wild canids, especially in young animals (Seguel and Gottdenker, 2017).

Roundworms (Nematodes belonging to the family Ascarididae) are larger intestinal parasites, typically 10-15 cm in length as adults, and known to infect most carnivorous species including coyotes with *Toxocara canis* found frequently in canid species. The adult worms residing in the intestines reproduce and release ova which are spread to the soil through the infected host's feces. The amount of time taken for ova to mature to the final larval stage (L3) ranges from 2 to 6 weeks (Otranto et al, 2015), with the L3 larvae still contained in the ovum's shell (Roberts et al Ch. 26, 2013). Depending on the species of parasite, the larvated ova typically remain infective in the environment for approximately 6 months, with some *Toxocara* spp. remaining viable for years (Mizgajska-Wiktor, 2017). The L3 larvae are ingested by a host, where they are transported to the liver and lungs once they have burrowed through the intestinal walls, and eventually migrate to the trachea, where they are coughed up and swallowed before migrating to the intestines and attaching as adult worms (Otranto et al, 2015). In addition to direct consumption of ova from the environment, canines are also able to contract *Toxocara canis* from eating infected rodents, which act as a paratenic host to the parasite by harboring larval stages (visceral larva migrans) of a parasite with no development of the adult stages (Schantz, 1989). Puppies are typically infected with *Toxocara canis* from the time of birth, as the parasite can be transferred transplacentally from mother to offspring (Gillespie, 1989). *Toxocara* spp. adults divert nutrition from their hosts, resulting in poor nutrient utilization, and gastrointestinal upset including nausea and vomiting. *Toxocara canis* also poses a risk to humans as it is the most common of several nematodes that act as a causative agent of Visceral Larva Migrans (Roberts et al Ch.26, 2013). When larval worms are ingested by an organism that is not a natural host to the nematode, the larval (juvenile) stages of the parasite can migrate through organs, or viscera, of the body, mostly ending up in the brain and liver where they manage to evade the host's immune defenses (Schantz, 1989). Those with visceral larva migrans display pulmonary symptoms, fever, enlarged liver, a high eosinophil count, neurological symptoms, motor dysfunction, and death in cases of heavy infections in the brain (Schantz, 1989).

Whipworms (Nematodes belonging to the Family Trichuridae) are intestinal parasites of both humans and carnivorous mammals. The adult worms range from 4.5 to 7.5cm in length, with a "hair-like" or "whip-like" anterior extension (Traversa, 2011). Adult worms reside and reproduce in the small intestine, and release oval-shaped ova with bilateral plugs, which pass with the host's feces into the environment, where the ova flourishes in moist, shaded soil, and embryonates to the L1 larval stage in approximately 21 days (Roberts et al Ch. 23, 2013). Once the ova are ingested by a host, the larvae hatch and penetrate the intestinal wall and mature to adulthood over a period of approximately 75 days (Roberts et al Ch. 23, 2013). The anthroponotic *Trichuris trichiura* is the primary species associated with infection in humans; however, *Trichuris vulpis*, which is predominately found in carnivorous canids has been tentatively identified as a zoonosis in multiple human cases (Kenney et al,). While diagnosis of humans with *Trichuris vulpis* was based on morphological differences between *Trichuris trichiura* and *Trichuris vulpis* ova and adults, a definitive diagnosis with genetic analysis has yet to prove the infectivity of *Trichuris vulpis* to humans (Traversa, 2011).

Fecal analysis of free-ranging coyotes located at the Savannah River Site along the South Carolina/Georgia border found *Trichuris* spp. ova in one of 41 fecal samples (Miller et al, 2009), and dissection of coyotes from the Southeast Nebraska/ Southwest Iowa region indicated presence of *Trichuris vulpis* in 3 out of 29 samples (Redman et al, 2016). Analysis of fecal samples in multiple European cities revealed that stray dogs had higher infection rates (60%) than those of pet dogs (2.6%-30%), indicating that strays may be responsible for spreading and maintaining the parasite in the environment (Traversa, 2011). This logic could also be applied to coyotes as transient populations roam in urban and suburban areas. Infection by *Trichuris vulpis* in domestic dogs is known to cause damage to the cecum (Burrows and Lillis, 1964), poor nutritional absorption in the host, and episodes of diarrhea (Traversa, 2011). Heavy infestations can cause hemorrhagic diarrhea, and sometimes pure blood passed through the anus, with the resulting blood loss causing weakness, lethargy, and anemia (Traversa, 2011).

In addition to intestinal nematodes, coyotes are also hosts for intestinal cestodes (Class Cestoda, Phylum Platyhelminthes), known commonly as tapeworms. Tapeworms are a large and diverse group capable of infecting many species of the animal kingdom. Consisting of a scolex (head) and many segments known as proglottids, adult tapeworms are hermaphroditic with proglottids containing both male and female reproductive organs (Roberts et al, Ch. 20, 2013). Adult cestodes reside in the intestines of their definitive host, and release gravid (egg containing) proglottids which usually pass with feces, but are mobile and capable of maneuvering their way out of their host's anus on their own (personal observations at veterinary clinic). These proglottids act as egg packets, housing a large number of ova which are released into the environment and ingested by an intermediate host (Roberts et al Ch. 21, 2013). The genera of tapeworms vary in the way they reside in the intermediate host from plerocercoids (*Diphyllobothrium* spp.), cysticercoids (*Taenia* spp. and *Dipylidium* spp.), and hydatid cysts (*Echinococcus* spp.); however, it is universal that the definitive host encounters the parasite by ingesting the intermediate host (Roberts et al, Ch. 21 2013).

The most commonly identified tapeworms in coyotes include *Taenia pisiformis*, *Taenia hydatigena*, *Echinococcus granulosus, Echinococcus multilocularis, Dipylidium caninum,* and *Hymenolepis diminuta* (Redman et al, 2016; Schurer et al, 2018). Fecal samples analyzed from coyotes in the neighboring state of Florida found coyotes to host the following cestodes: *Diphyllobothrium latum, Dipylidium caninum, Hymenolepis spp.,* and *Taenia spp.* (Grigione et al, 2014). While coyotes are not capable of directly transmitting cestode infections to domestic dogs due the requirement of an intermediate host for parasite development, coyotes do serve as a method of transport and dispersal of gravid proglottids. Defecation in the vicinity of residences and areas frequented by domestic animals helps to maintain the prevalence of cestode infections in intermediate hosts in those areas. One concern for humans who encounter coyote feces (usually through infected water or food plants which have not been properly washed) is the ova of the zoonotic tapeworm species, *Echinococcus granulosus and Echinococcus multilocularis* (CDC,

2012). When the ova are ingested by an accidental intermediate host, like humans, they hatch into oncospheres which travel out of the intestines by penetrating the intestinal wall, and then migrate throughout the body to areas like the lungs, liver, and even the brain (CDC, 2012). The oncosphere then produces a hydatid cyst (CDC, 2012) which can develop over a period of several years up to a decade (Roberts et al Ch. 21, 2013). The consequences of infection with a hydatid cyst, known as cystic echinococcosis, are serious, with the possibility of death due to serious anaphylactic reactions if a cyst ruptures, and surgical aspiration or removal of cysts being the only method of treatment (CDC, 2012). Infection with *Taenia* spp. tapeworms in mammals can result in decreased nutrient absorption in the host due to the tapeworm's ability to absorb nutrients through its tegument (Roberts et al, Ch.21, 2013).

This study analyzed the contents of coyote intestines for adult parasites and parasitic ova in fecal flotation in order to assess the species diversity and distribution of intestinal parasites in a representative sample of Georgia's coyote population.

MATERIALS AND METHODS

Collection of Organs

Coyotes were positioned in dorsal recumbency on a dissection table or on the tailgate of a truck in the case of field dissections. A piece of rope was attached to each limb and tied a stationary object to stabilize the coyote throughout dissection, similar to how canine patients are positioned and secured to the operating table during surgery.

Dissection of the coyote was performed using sharp/ blunt straight surgical scissors and a lockback-skinner knife, which was disinfected with an aqueous 10% bleach solution and manual scrubbing with a brush and paper towels between each specimen. A deep incision was made along the ventral midline through the entirety of the sternum extending posteriorly to the diaphragm to access and remove the heart, then extended down the midline from the diaphragm to the posterior of the ventral abdomen. The greater mesentery was removed to access the intestinal tract. All efforts were made to resect the entirety of the small intestine and majority of the large intestine (ending at the colon), however in some cases bullet wounds to the abdomen and friability of the intestines only allowed for a smaller portion of the intestinal tract to be resected. A ligature of twine was used to tie off the small intestine between the pylorus and duodenum, and a cut was made on the pyloric side of the ligature. A ligature was placed on the large intestine on the descending colon as close to the anus as possible, and cut on the anal side of the ligature (Figure 3.1). The intestines were detached from the body by cutting the omentum and peritoneum, then placed in a Ziploc bag with 70% ethanol to preserve for later evaluation.

Figure 3.1: Removal of intestines from coyote during a field dissection.

Evaluation of intestines for parasites

Preserved intestinal samples were transported to the lab, and stored at 4ºC until examination. Resected intestinal segments were then placed in a large tray and the mesentery was removed. A longitudinal incision was made down the entire length of intestine using tissue forceps and dissection scissors. The intestines were rinsed with a 0.9% solution of saline to help separate ingesta from helminths, and a glass microscope slide was used to scrape the inner wall of the intestine to detach any parasites still attached to the intestinal wall. The contents of the intestines were added to a large beaker of 0.9% saline and allowed to separate. The supernatant was removed by pipette and examined by a dissecting microscope. The material which had settled to the bottom was then examined by a dissecting microscope. The contents of the intestines were examined

thoroughly, and all helminths placed in containers with the appropriate preservative, nematodes and acanthocephalans in 70% ethanol with 5% glycerin and cestodes in 70% ethanol. Cestodes were identified to the highest possible taxonomic level using information from Chapters 20 & 21 of "*Foundations of Parasitology 9th edition"* (Roberts et al, 2013)*,* "*Pictorial Key to Families of Cestodes of Veterinary Importance*" published by Oklahoma State College of Veterinary Health Sciences, and data on morphological and host-specificity of cestode families.

Evaluation of fecal samples

A fecal sample was removed from the large intestine and a fecal flotation test was performed using a zinc sulfate solution with a specific gravity of 1.18 in an 8 dram pill vial. Samples from the fecal float were observed through a compound microscope at 100X to 400X magnification and ova were identified to the most specific taxonomic level possible. If there were any adult parasites that were not detected through manual dissection due to their absence in the section of intestine resected from the coyote, the presence of ova in the fecal float sufficed as an indicator of infestation by that parasite.

Statistical analysis

Analysis of variance (ANOVA) testing was conducted to determine the effects of approximate age, sex, DNR management region of origin, and geographic region of origin on both species richness per coyote and number of adult parasites found per coyote. Bivariate analysis with a line of fit was used to analyze the effect of approximate weight on species richness and number of adult parasites found per coyote.

RESULTS AND DISCUSSION

A total of 38 sets of intestines from coyotes were examined for the presence of both adult intestinal parasites and their ova, with 30 specimen (78.95%) having at least one life stage of parasite present in the intestines. Both ova and adult parasites were present in 15 specimen (39.47%), adult parasites present in 20 specimen (52.63%), ova present in 23 specimen (60.52%), and no adults or ova present in 8 specimen (21.05%).

A total of 313 adult parasites were collected from all geographic and DNR management regions: 193 *Ancylostoma* spp., 116 *Taenia* spp., 2 *Physaloptera* spp., 1 *Ascaris* spp., and 1 *Macranthorhynchus* spp. (Table 3.1 and Table 3.2). Fecal flotation indicated infection with *Ancylostoma* spp. in 23 specimens, *Trichuris vulpis* in 2 specimens, *Uncinaria stenocephala* in 2 specimens, and *Passalurus* spp. in 1 specimens (Figure 3.2 and Figure 3.3).

Approximate age had a noticeable trend in relation to the species richness of intestinal parasites, with adult coyotes aged 1-3 years having the highest species richness, while sex, approximate weight, and region of origin showed no correlation (Table 3.3). None of the factors tested showed correlation in regards to the number of adult parasites found (Table 3.4).

Table 3.1: Occurrence of intestinal parasites by DNR management region of origin.

DNR Region	Parasites present
Northeast	*Ancylostoma* sp. *Macracanthorhynchus* sp. *Taenia* spp. *Trichuris vulpis*
Northwest	*Ancylostoma* sp. *Taenia* spp. *Trichuris vulpis*
East Central	*Ancylostoma* sp. *Taenia* spp.
West Central	*Ancylostoma* sp. *Taenia* spp.
South Central	*Ancylostoma* sp. *Passalurus* sp. *Physaloptera* sp. *Taenia* spp.
Southwest	*Ancylostoma* sp. *Physaloptera* sp. *Uncinaria* sp. *Taenia* spp.

Table 3.2: Occurrence of intestinal parasites by geographic region of origin.

Geographic Region	Parasites present
Ridge and Valley	*Ancylostoma* sp. *Taenia* spp. *Trichuris vulpis*
Piedmont	*Ancylostoma* sp. *Macracanthorhynchus* sp. *Taenia* spp. *Trichuris vulpis*
Upper Coastal Plains	*Ancylostoma* sp. *Physaloptera* sp. *Uncinaria* sp. *Taenia* spp.
Lower Coastal Plains	*Ancylostoma* sp. *Passalurus* sp. *Taenia* spp.

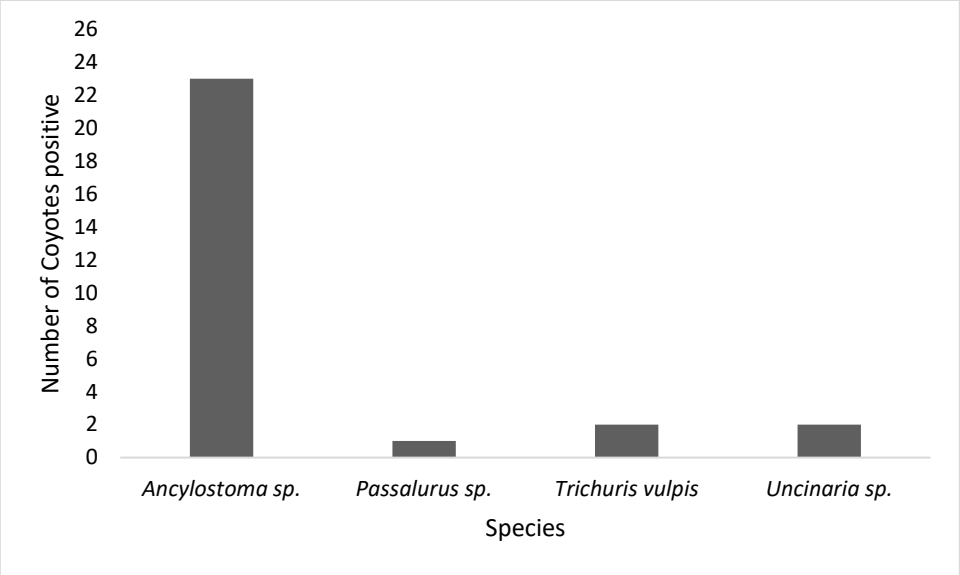

Figure 3.2: Frequency of parasitic ova present in coyotes found in fecal flotation.

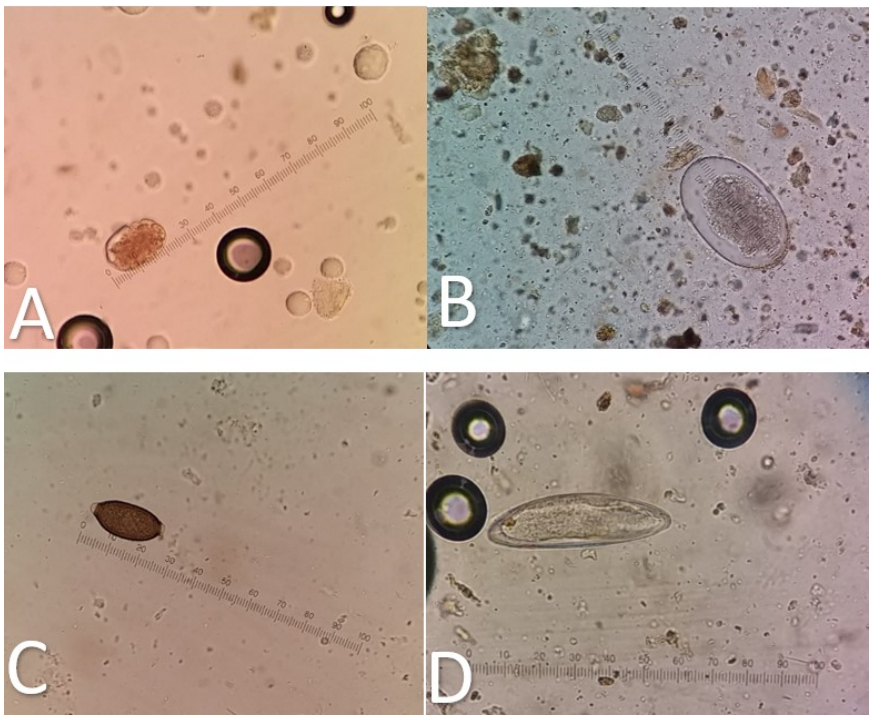

Figure 3.3: Species of parasitic ova found using fecal floatation include *Ancylostoma* sp. (A), *Uncinaria stenocephala* (B), *Trichuris vulpis* (C), and *Passalurus sp.* (D) photographed at 400X.

Table 3.3: Statistical analysis of factors affecting species richness of intestinal parasites found in coyotes.

Factor	Sample size (n)	Degrees of freedom (df)	Levene's P	Prob>F
Approximate Age	38	3	0.0219	0.0450
Approximate Weight	38	1	N/A	0.1919
Sex	38	1	0.0234	0.8717
DNR management region	38	5	0.1041	0.1475
Geographic region	38	3	0.262	0.3473

Table 3.4: Statistical analysis of factors affecting number of adult intestinal parasites found in coyotes.

Factor	Sample size (n)	Degrees of freedom (df)	Levene's P	Prob> F
Approximate Age	38	3	0.4541	0.7507
Approximate Weight	38	1	N/A	0.1292
Sex	38	1	0.0437	0.2264
DNR management region	38	5	0.0195	0.6390
Geographic region	38	3	0.1661	0.8390

Manual examination of intestines for adult parasites combined with testing by fecal floatation for parasite ova found 30 of 38 coyotes (78.95%) to be infected by intestinal parasites. The higher success rate of diagnosing parasitism by fecal flotation (60.52%) vs manual dissection (52.63%) indicates the importance of using both methods concurrently to avoid false negative reports. There were 8 specimen (21.05%) in which neither adult parasites nor ova was found, for 4 of these specimen it was noted on the dissection sheet that there was an extremely small segment of intestines resected due to bullet wounds penetrating the intestines, which indicates the importance of fully resecting the intestines.

Ancylostoma spp. and *Taenia* spp. were found in every region (DNR/geographic), with *Trichuris vulpis* confined to the Northeast and Northwest/ Piedmont and Ridge and Valley Region,

Macracanthorhynchus spp. occurring in only the Northeast/ Piedmont region, *Uncinaria stenocephala* confined to the Southwest/ Upper coastal Plains region, and *Physaloptera* confined to the South Central and Southwest/ Upper Coastal Plains region.

The most frequently found adult parasites were tapeworms belonging to the genus *Taenia;* of the 21 coyotes from which adult intestinal parasites were collected from, 15 coyotes were infected with *Taenia* spp. tapeworms. Tapeworms were identified to the genus *Taenia* based on the morphological characteristics of both the scolex, mature proglottids, and ova found in gravid proglottids (Figure 3.4, Figure 3.5, and Figure 3.6). While the genus *Taenia* is quite diverse and accurate species identification is difficult to determine without the use of DNA sequencing, the tapeworms collected are mostly believed to be *Taenia pisiformis*, *Taenia serialis, or Taenia hydatigena* based on morphological characteristics, previously reported incidences of definitive hosts, range, and the role of organisms which coyotes eat as intermediate hosts. *Taenia pisiformis* intermediate hosts include rabbits, *Taenia serialis hosts* include rabbits and squirrels, and *Taenia hydatigena* hosts include cattle, sheep, goats, pigs, with rabbits and rodents occasionally being infected (Merck Veterinary Manual, 2019).

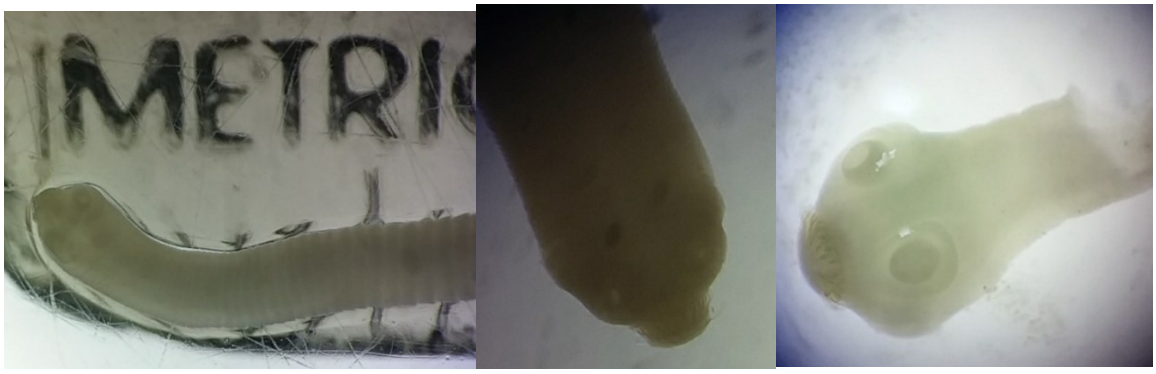

Figure 3.4: Scolices of *Taenia* spp. Tapeworms collected from coyotes, believed to be *Taenia pisiformis*.

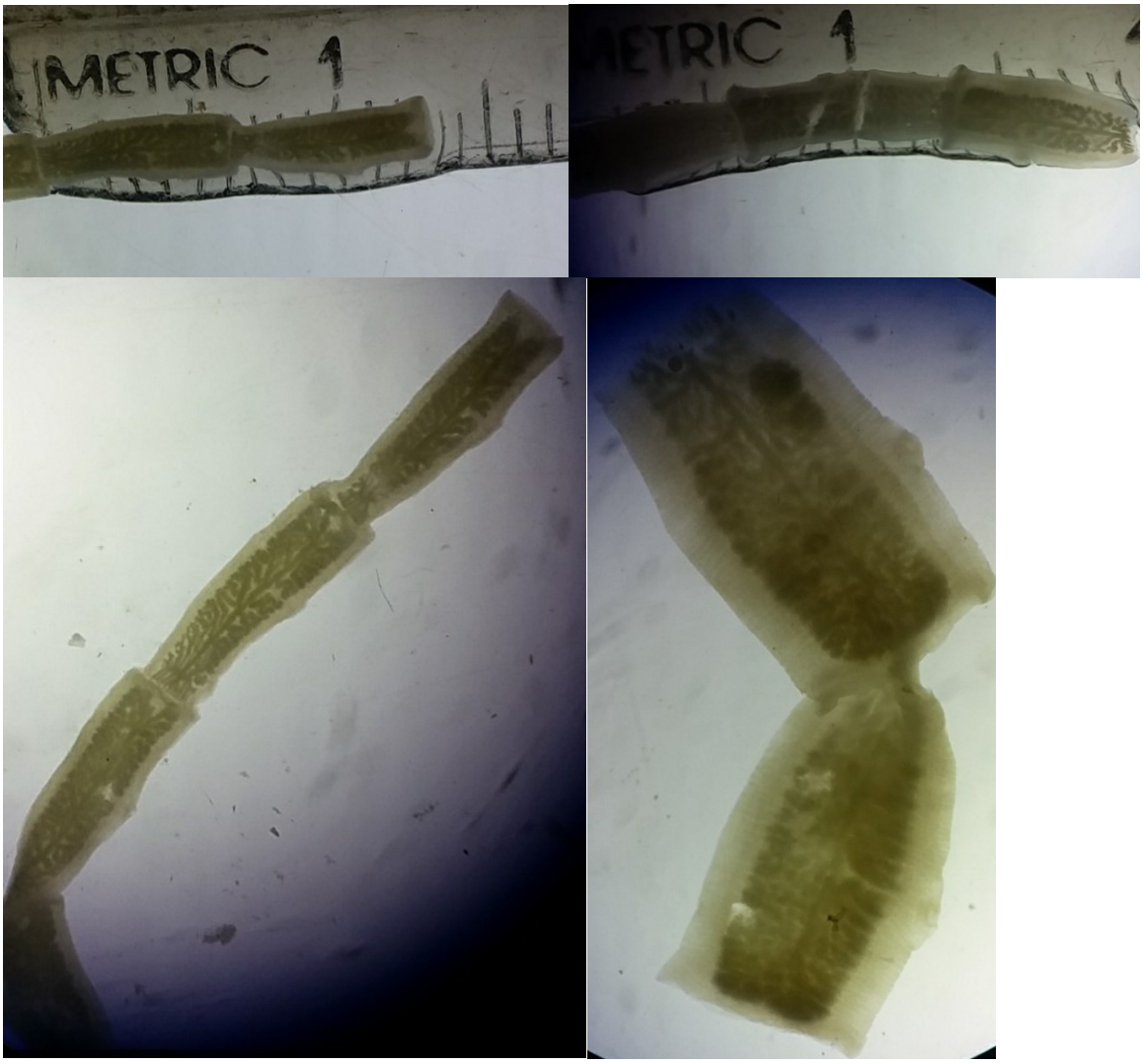

Figure 3.5: Proglottids from 4 tapeworms collected from separate coyotes.

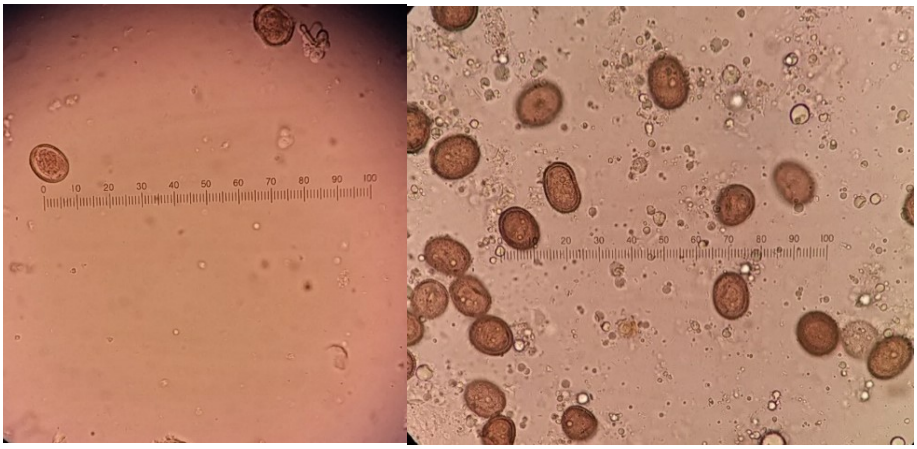

Figure 3.6: Ova from gravid proglottids collected from separate coyotes, pictured at 400X.

Ancylostoma spp. adults were another commonly found adult parasite, with burdens ranging from 1 to 175 adult worms. Based on morphlogical characteristics (Figure 3.7), the specimens are likely *Ancylostoma caninum;* however, it is difficult to accurately distinguish between *Ancylostoma caninum* and *Ancylostoma braziliense* without DNA sequencing.

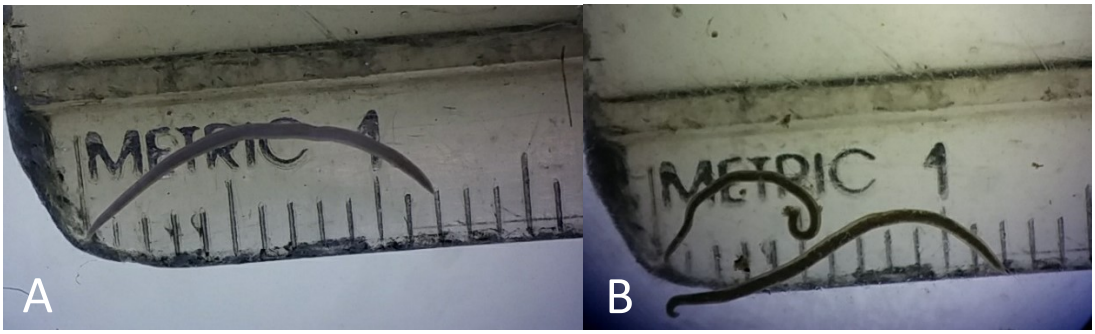

Figure 3.7: Images of *Ancylostoma* sp. collected from separate coyotes, females are larger (on bottom in image B).

One Acanthocephalan worm, tentatively identified as *Macracanthorhynchus* sp. based on morphological characters (Figure 3.8), was found in a coyote from the Piedmont/ Northeast region (Gwinnett County), and is the first reported occurrence of an acanthocephalan worm in Georgia's coyote population. Both *Macracanthorhynchus ingens* (primarily found in raccoons) and *Macracanthorhynchus hirudinaceus* (primarily found in swine) are infrequently reported in canids, therefore making it hard to identify by host association (CAPC, 2018). The specimen recovered is not as long as stated in species descriptions, however it was not relaxed at the time of measurement. Acanthocephalan worms are primarily found in wildlife hosts at lower frequencies than Cestodes and Nematodes, and are generally not found in domestic animals (CAPCA, 2018). *Macracanthorhynchus ingens* was found to cause bloody, loose stools in puppies (Fahnestock, 1985), and *Macracanthorhynchus hirudinaceus* was found to cause slowed growth and emaciation in swine (CAPC, 2018).

Figure 3.8: *Macracanthorhynchus* sp. removed from the intestine of a coyote from Gwinnett Co., GA both magnified and with ruler for scale (approximately 5mm in length).

An adult stomach worm, *Physaloptera* sp. was found in a coyote from the South Central/ Upper Coastal Plains region (Treutlen County). Identification was based on morphological characteristics (Figure 3.9) and previous records of *Physaloptera* spp. in coyotes (Bridger et al, 2009). Infection with *Physaloptera* spp. can cause gastritis resulting in vomiting, anorexia, and dark feces (from the presence of digested blood), and the worms moving attachments sites in the stomach can damage the gastric mucosa, leading to anemia and weight loss (Peregrine, 2019).

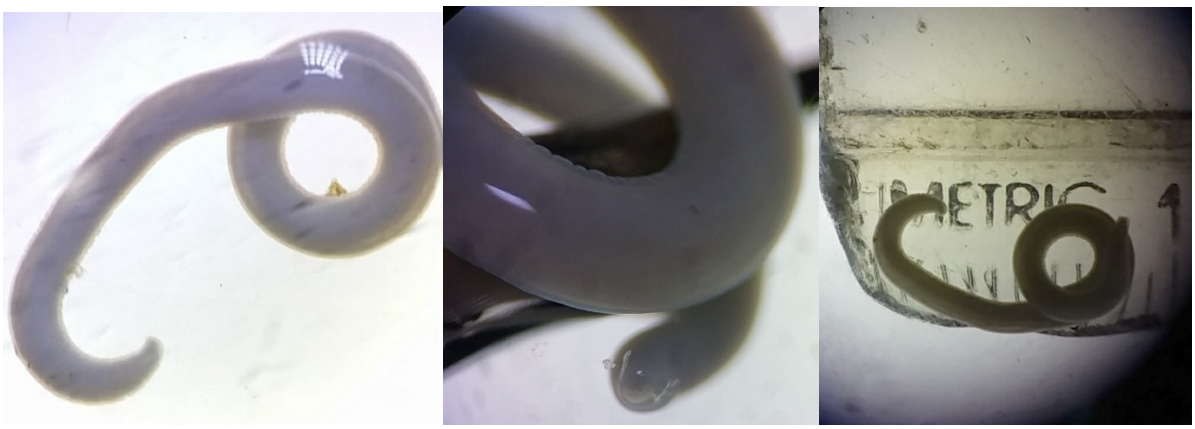

Figure 3.9: Images of *Physaloptera* sp. worm collected from coyote taken from various angles.

The most commonly occurring parasite found on fecal flotation was *Ancylostoma* sp., occurring in 60.52% of specimen, which is consistent with the range of *Ancylostoma* spp. infection found in fecal flotation from other recent studies in the Southeastern Unites States: 20-24% in Florida (Grigione et al, 2014), 37% in central Georgia (Gates et al, 2014) and 93% in North Carolina (Chitwood et al, 2015). Two instances *of Uncinaria stenocephala* were noted (5.26%), similar to the frequency found in a study from Florida (5.0%) (Grigione et al, 2014). *Trichuris vulpis* was also found in only 2 specimen (5.26), resulting in a lower frequency than previous works in central Georgia (20%) (Gates et al, 2014), Florida (32.5%) (Grigione et al, 2014), and North Carolina (29%) (Chitwood et al, 2015). There was one incidence of suspected *Passalurus* spp. (Rabbit pinworm) ova found in a specimen from the South Central/ Lower Coastal Plains region, which is not consistent with results from local studies. However due to the parasite's typical lifecycle utiliziing lagomorphs as hosts, and the coyote's exploitation of rabbits as a food source, there is a possibility for coyotes to become infected with *Passalurus* spp., which has historically been documented in coyotes in Utah (Butler and Grundmann, 1954).

Overall, the species of intestinal parasites found in this survey were mostly consistent with previous studies in Georgia and surrounding states. Some intestinal parasites collected in this study such as *Physaloptera* spp. and *Macracanthorhynchus* spp. have not yet been reported in recent studies of intestinal parasites of coyotes in the state. Based on the intestinal parasites found, coyotes throughout Georgia play a role as wildlife hosts of nematodes and cestodes of medical and veterinary importance. Through traveling to hunt or in search of a home territory or range expansion, coyotes roam in many different habitats of Georgia, more recently including suburban and urban areas in addition to their typical rural range. As a coyote's range expands, the likelihood of it depositing feces in areas proximate to human and domestic pet's residences increases. Domestic pets coming into contact with soil contaminated by coyote feces introduces the risk for infection with *Ancylostoma* spp. (hookworms), and *Trichuris vulpis* (whipworms). In the case of *Taenia* spp. (tapeworms), the coyotes' feces is infective to intermediate hosts such as rodents, hares, and ungulates, which can later be ingested by domestic dogs.

Similar to tapeworms, both *Physaloptera* spp. (CAPC, 2018) and *Macracanthorhynchus* spp. (CAPC, 2018) parasitize invertebrates as intermediate hosts, which can be infected by ingesting coyotes' feces and may be later ingested by domestic dogs. While the presence of roundworms (*Toxocara* spp.) or species of tapeworms detrimental to humans such as *Echinococcus granulosus* or *Echinococcus multilocularis* were not found in this survey, there is a likelihood that these parasites are present in Georgia's coyote population due to their discovery in coyotes from a study in the neighboring state of Florida (Grigione et al, 2014).

In order to better understand the intestinal parasites infecting coyotes, all parasites collected will need to be identified to species, which is an option for expansion upon this initial project. Also, a larger sample size, preferably from coyotes whose intestines have not been compromised by a shot to the abdomen, would help to yield a greater number of intestinal parasites collected.

CHAPTER 4
ECTOPARASITES AND TICK-BORNE DISEASES

Tick species and Tick-Borne illnesses affecting Canis latrans, domestic canines, and humans

Ticks are hematophagous ectoparasitic arachnids belonging to the Order Acari which are distributed throughout the world (Magnarelli, 2009), and found in all 50 United States (Nieto et al, 2018). Three families of ticks exist: Ixodidae- hard ticks, Argasidae- soft ticks, and Nuttalliellidae (Guglielmone et al, 2010); however, this study only focused on members of the family Ixodidae due to their presence in the study area and role as vectors of pathogens.

The ixodid tick lifecycle begins with copulation, which can occur while the male and female ticks are on or off a host organism (Roe and Sonenshine, 2014). Once the female is engorged with blood, she will detach from the host, lay eggs (average of a few thousand) in soil or detritus, and die in a matter of days (Roe and Sonenshine, 2014). Ova typically hatch in a matter of 2-3 weeks (Roe and Sonenshine, 2014); however, in cooler climates the eggs may overwinter before hatching. When larvae emerge from the ova, they migrate to vegetation and begin a behavior known as questing, in which they use their sensory Haller's organ located on the first tarsi to detect carbon dioxide, humidity, and heat released by a potential host (Mullen and Durden Ch. 26, 2009). Tick species of interest to this study include the genera *Ixodes, Dermacentor,* and *Amblyomma*, all of which have 3-host life cycle, in which each life stage feeds on a different vertebrate host (Roe and Sonenshine, 2014). Larval ixodid ticks will feed on a host, engorge, drop off, and molt to a nymph which quests for a host, feeds to engorgement, drops off, and undergoes a final molt to an adult which will then quest, feed, and reproduce (Roe and Sonenshine, 2014). A survey of ticks present on wildlife in various locations throughout Florida found coyotes to be hosts of *Amblyomma americanum, Amblyomma maculatum*, *Dermacentor variabilis,* and *Ixodes scalpularis* (Hertz et al, 2017), with all 4 species acting as competent vectors of numerous zoonotic diseases.

Amblyomma americanum, the Lone Star Tick, is one of the most frequently found ticks in the Eastern United States (Merten and Durden, 2000), and Southeastern United States (Biggs et al, 2016) and is a competent vector of multiple disease-causing organisms including pathogenic *Rickettsia* and *Ehrlichia* bacteria as well as viral diseases such as Heartland Virus.

Ehrlichioses are diseases caused by *Ehrlichia* spp. bacteria known to cause severe headache, nausea, vomiting, muscle aches, and progress if not treated to cause encephalitis, respiratory failure, and organ failure, all of which can be fatal (CDC, 2019). Although the name Human Monocytic Ehrlichiosis (HME) leads one to believe that the disease is an anthroponosis, wildlife such as white-tailed deer and coyotes can also be infected by the causative agent of HME*, Ehrlichia chaffeensis* and act as reservoir hosts (Davidson et al, 2001; Kocan et al, 2000). In a study of coyotes from Oklahoma, 15 of 21 (71%) tested positive for *E. chaffeensis*, the causative agent of HME, while interestingly no coyotes tested positive for *Ehrlichia canis*, the causative agent of Canine Monocytic Ehrlichiosis or *Ehrlichia ewingii* , the causative agent of Canine Granulocytic Ehrlichiosis (Kocan et al, 2000). While the coyotes from the study in Oklahoma were not found to be infected with *E. canis*, they are competent hosts and have been experimentally infected with *E. canis* (Ewing et al, 1964), but it should be noted that *A. americanum* has not been proven as a competent vector for *E. canis* (Stitch et al, 2008). Blood tests from a home with both canine and human family members testing positive *for E. chaffeensis, E. ewingii,* and *Anaplasma platys* indicates the potential risks that interactions with canines poses to humans (Breitschwerdt et al, 2014).

Francisella tularensis, the causative agent of Tularemia is another pathogenic bacterium capable of transmission to humans and animals through the bite of infected ticks, typically *A. americanum* (Brown et al, 2011). In Nebraska, 32% of coyotes in a serological survey tested positive for antibodies against *F. tularensis* (Bischof and Rogers, 2005). Tularemia symptoms in humans include fever, chills, and myalgia which can progress to pneumonia, sepsis, and death (AVMA, 2003). Clinical symptoms of Tularemia in canines are rarely seen, but can include fever, anorexia, and nasal discharge (AVMA, 2003). *Francisella*

tularensis can also be transmitted to humans through the bite of an infected animal, with cases of Tularemia after an infected coyote bite being reported recently in Montana and California (Chomel et al, 2015).

Southern Tick Associated Rash Illness (STARI) is an anthroponotic disease characterized by an erythema migrans rash and fatigue, and is caused by an undetermined bacterium transmitted through the bite of *A. americanum* ticks (Wormser et al, 2005). Finally, *A. americanum* has been identified as a vector of the Heartland Virus (HRTV), which is an emerging disease initially diagnosed in human patients in the Midwestern region of the United States in Missouri in 2009 (Brault et al, 2018). The 33% transovarial infection rate in experimentally infected *A. americanum* females suggests that transovarial transmission may be possible in the wild as well (Brault et al, 2018). Serosurveillance of wildlife across 13 Midwestern and Southeastern States revealed 11 of 69 (16%) of tested coyotes to have HRTV neutralizing antibodies, indicating exposure to the virus (Riemersma and Komar, 2015).

Gulf Coast ticks, *Amblyomma maculatum,* collected from vegetation in Georgia (Bulloch and Macintosh counties), and a coyote in South Carolina (Anderson county) tested positive for the obligate intracellular pathogenic bacterium *Rickettsia parkeri* (Sumner et al, 2007). Infection with *R. parkeri* can cause a disease similar to Rocky Mountain spotted fever known generally as "*Rickettsia parkeri* Rickettsiosis", which can affect humans (CDC, 2019; Sumner et al, 2007). While domestic dogs from shelters in various areas of Louisiana were seropositive for *R. parkeri*, none actively showed signs of Rickettsiosis at the time of testing (Grasperge et al, 2011). Many ticks are known to be capable of passing pathogens through vertical transmission, also called transovarial transmission (mother to offspring), and transtadial transmission (transmission from one life stage to the next, remaining infected through a molt). In *A. maculatum* ticks experimentally infected with *R. parkeri*, transovarial transmission was effective in 88% of cases, and transtadial transmission was effective in 100% of cases, indicating that infection with *R. parkeri* does not have a negative effect on transmission from an infected mother to its offspring and the offspring's subsequent life stages (Wright et al, 2015). This also indicates that *A.*

maculatum can be born with *R. parkeri* in its system and does not require feeding on a host to become infected. Furthermore, an *A. americanum* tick removed from a coyote in Knox County, Tennessee tested positive for *R. parkeri,* which could have been circulating in the bloodstream of the coyote and ingested by the tick during its blood meal, or the *R. parkeri* could be transmitted by *A. americanum* ticks (Cohen et al, 2009).

Dermacentor variabilis, the American Dog tick, is another tick native to the Southeastern United States (Merten and Durden, 2000). Nymphal stages of *D. variabilis* have been found to serve as vectors of Tularemia in addition to *A. americanum*; however, the cost of infection is high to the tick resulting in low transmission rates (Reese et al, 2010). *D. variabilis* and *Rhipicephalus sanguineus* are associated with the transmission of both *Ehrlichia canis,* the causative agent of Canine Monocytic Ehrlichiosis discussed previously, and *Anaplasma platys*, the causative agent of Canine Anaplasmosis, more specifically Canine Cyclic Thrombocytopenia (CVBD, 2017). Infection with *A. platys* in canines causes cyclical fever and thrombocytopenia- low platelet count (Gaunt et al, 1990). Rocky Mountain spotted fever (RMSV), caused by the bacterium *Rickettsia rickettsii*, is the disease most commonly associated with *D. variabilis*, proven to be a competent vector of *R. rickettsii* capable of transovarial and transtadial transmission(Harris et al, 2017). Studies of *D. variabilis* experimentally infected with *R. rickettsii* revealed no significant cost to the tick aside from lower hatch success rates in eggs (Schumacher et al, 2016). Additional vectors of RMSF include *Dermacentor andersoni*, which is native to the Western United States, and *R. sanguineus,* with the only reported RMSF cases associated with *R. sanguineus* occurring in Arizona and along the Mexico/U.S. border (Biggs et al, 2016). Human cases of Spotted Fever Rickettsiosis were reported in approximately 75% of Georgia counties between 2000 and 2013 (Biggs et al, 2016). RMSF is a potentially dangerous disease in humans, with initial symptoms including fever, chills, fatigue, headache, myalgia, nausea, vomiting, and a maculopapular rash that may spread across the body. If the infection is not treated early, it can progress and cause more severe symptoms, many of which are life threatening, including meningoencephalitis, acute renal failure, acute respiratory

distress, cutaneous necrosis, shock, arrhythmia, and seizures (Biggs et al, 2016). Symptoms of RMSF in dogs include fever, anorexia and abdominal pain, fluid retention (edema) in extremities, petechiae on mucosal membranes, myalgia, arthritis, confusion, head tilt, circling, nystagmus, cough, and dyspnea (Greene and Breitschwerdt, 1998). According to serological surveys, 13% of coyotes from Nebraska and 60% of coyotes tested from sites in Oklahoma and Texas were found to have anti- *R. rickettsii* antibodies in their bloodstream (Bischof and Rogers, 2005; Starkey et al, 2013).

The final tick commonly associated with coyotes in the Southeastern United States is *Ixodes scapularis*, commonly found on vertebrate hosts in the state of Georgia (Merten and Durden, 2000), and found in the entire Eastern half of the United States (Biggs et al, 2016). It is a vector of several agents, including those that cause two diseases relevant to the species in this study: Lyme disease and Anaplasmosis. Anaplasmosis is a general term for diseases caused by bacteria belonging to the genera *Anaplasma,* including *Anaplasma platys* which infects canines (transmitted by *D. variabilis* and *R. sanguineus*), and *Anaplasma phagocytophilum* (transmitted by *I. scapularis*) which infects humans (Biggs et al, 2016). Canines can also serve as opportunistic hosts of *A. phagocytophilum* (Bowman et al, 2008). *A. phagocytophilum* infection causes headache, fever, chills, malaise, and myalgia, with rare occurrences of rash, gastrointestinal symptoms, or neurological symptoms. More serious cases can mimic Toxic Shock Syndrome and involve acute respiratory distress syndrome, neuropathy, rhabdomyolysis, pancreatitis, acute renal failure, and hemorrhaging/ coagulopathy due to the low platelet count (Biggs et al, 2016).

Coyotes, deer, and mountain lions have not been proven to be active carriers of the same strain of *A. phagocytophilum* which infects domestic canines and humans (Foley et al, 2008); therefore, wildlife may only play a role in the transmission of *A. phagocytophilum* to humans by transportation and dispersal of infected *I. scapularis* ticks. It is also worth noting that despite the distribution of *I. scapularis,* the incidence of infection by *A. phagocytophilum* in domestic canines is not as high in the Southeastern

United States (0.5% positive) as it is in other regions such as the Midwest (5.5% positive) (Bowman et al, 2008).

In addition to Anaplasmosis, *I. scapularis* is the vector for the more commonly known condition called Lyme disease. Caused by the spirochete bacterium, *Borrelia burgdorferi* sensu strictu, the Lyme disease transmission cycle begins with *I. scapularis* larvae feeding on a small host like a bird or rodent (CDC, 2019). In the Southeastern United States, the rodent species *Peromyscus gossypinus* (White-Footed Mouse), *Sigmodon hispidus* (Hispid Cotton Rat), and *Neotoma floridana* (Eastern Woodrat) are common hosts to larval *I. scapularis*, and are all known reservoir hosts of Lyme disease (Oliver et al, 2003). Once a larval tick has engorged, dropped off the host, and molted to a nymph, it begins questing for the next host, which can include *Odocoileus virginianus* (White-Tailed Deer), Humans, and other mammals including canids (CDC, 2019). The adult stage of *I. scapularis* prefers the White-Tailed deer as a host for copulation and as a feeding source (Kilpatrick et al, 2014). Because White-Tailed deer are incompetent hosts of *B. burgdorferi* s.s. and do not circulate the bacteria in their bloodstream (Telford et al, 1988), it is thought that larval ticks feeding on deer in place of rodents (reservoirs *for B. burgdorferi* s.s. can break the transmission cycle and result in less cases of Lyme disease in incidental hosts (Huang et al, 2019) such as humans and canids.

Exposure to Lyme disease analyzed in a multi-year survey by ELISA testing (SNAP 4dx) in domestic dogs is most prevalent in the Northeastern United States, the region where the disease was first discovered, with 11.6% of dogs testing positive for antibodies against *B. burgdorferi*, while only 1.0% of dogs tested positive for exposure from the Southeastern region, and 0.3% from Georgia (Bowman et al, 2009). Although limited data on coyote seroprevalance of *B. burgdorferi* in coyotes from local regions exists, ticks infected with *B. burgdorferi* were collected from a coyote in Canada (Smith et al, 2019). A more historical serosurvailence of coyotes in Texas for anti- *B. burgdorferi* antibodies revealed 0% prevalence from 1980-1983, followed by 48.5% in 1984, 22.7% in 1985, and 52.5 % in 1986 (Burgess,

1989). Human cases of Lyme disease reported to the CDC in 2017 included 3,483 confirmed cases and 2048 probable cases, with Georgia only accounting for 8 total cases which were confirmed (CDC, 2018).

The effects of Lyme disease on humans and canids are variable and not necessarily consistent with each other (Fritz and Kjemtrup, 2003). The initial symptoms of Lyme disease in humans include headache, fatigue, fever, chills, myalgia and arthralgia, swelling of the lymph nodes, and a distinctive erythema migrans rash which sometimes makes a bullseye shape as it increases in size (CDC, 2018). If treatment is not sought, the disease progresses, resulting in more severe headaches, spreading erythema migrans rash, severe swelling of joints, arthralgia, myalgia, neuralgia sometimes accompanied by numbness and tingling in extremities, ostealgia, arthritis, facial palsy, arrhythmias, dizziness, encephalitis, and problems with short term memory (CDC, 2018). Early treatment is available and usually curative; however, there are many instances of patients experiencing chronic symptoms of Lyme disease (CDC, 2018). In domestic dogs, veterinarians report the following symptoms: lameness 2-5 months after exposure, Arthritis typically in the tarsus or carpus, severe, often fatal renal disease, and occasional findings of myocarditis and neurological issues (Fritz and Kjemtrup, 2003).

A study on the spatial distribution of ticks found *Dermacentor* spp. to be 3 times more abundant and *Ixodes ricinus* to be 5 times more abundant in animal trails/ tracks than areas of vegetation 5 meters adjacent to the trails (Rasi et al, 2018). By acting as a host, coyotes are a mode of transportation for ticks, some of which serve as vectors for pathogenic bacteria. Because tick abundance is found to be greater along the direct path of a host animal, domestic pets or humans which reside in areas frequented by coyotes experience a higher likelihood of encountering a tick. Human infections with Rocky Mountain spotted fever were clustered in an area of Eastern Arizona with large populations of free-roaming dogs transporting *Rhipicephalus sanguineus* ticks (Demma et al, 2005). While vegetation and habitat are both important in predicting range and distribution of ticks, the role that hosts play in transport and dispersal of ticks is also an important factor in predicting tick population size and dispersal (Trout Fryxell et al, 2015). In conclusion, coyotes play a role in transporting and dispersing ticks which act as vectors in the

transmission cycles of various diseases. Because coyotes are often bitten by these infected ticks, they can become infected by certain pathogens and act as a reservoir of these tick-borne diseases in addition to a mode of transportation.

Mutual ectoparasitic insects of Canis latrans and domestic canines

While less serious than the diseases caused by ticks, coyotes also serve as hosts and modes of transportation for other ectoparasites such as fleas and lice, which are also hematophagous and can cause dermatitis and anemia in their host when present in high numbers (Mullen and Durden Ch. 9, 2009). Fleas can cause blood loss and dermatitis in their hosts and also serve as an intermediate host for the double-pored tapeworm, *Dipylidium caninum,* which can infect animals which ingest the infected flea (Mullen and Durden Ch. 9, 2009). *Ctenocephalides canis* (Dog Flea), and *Ctenocephalides felis* (Cat Flea), are found on dogs and cats as their name suggests, but also infest wildlife species as well, and have been found on 31 and 130 wildlife species, respectively (Clark et al, 2018). Adult fleas live on a host, copulate, and lay eggs: some of which remain on the host while others are spread to the host's environment, such as carpeting/ bedding indoors, or lawn areas outdoors. In order to be successful in an outdoor environment, flea eggs need to be in an area with 50% relative humidity and temperatures in the 4°C to 35°C range (Rust, 2005). There is speculation that coyotes and other wildlife introduce and maintain flea populations in environments near residential areas of domestic animals.

Trichodectes canis, the Canine Chewing Louse, has historically been found on coyotes in Alaska (Schwartz et al, 1983) and areas of Wisconsin and Minnesota (Mech et al, 1985), and is another mutual parasite of coyotes and domestic canines. Although irritating to the host, infestation with *T. canis* in coyotes and wolves did not seem to have an impact on host survival (Mech et al, 1985), however they are another known intermediate host of the tapeworm *Dipylidium caninum,* (Roberts et al Ch. 36, 2013) which could play a role in decreasing host fitness. Due to the role of *T. canis* in the *D. caninum* lifecycle, this louse poses risk to both canines and humans as both can be infected *by D.* caninum after ingestion *of T. canis* (Roberts et al, Ch. 36, 2013).

Purpose of this study

In addition to identifying and quantifying ticks present on the specimens used in this study, blood was collected (when able) and tested for the presence of antibodies against *Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Ehrlichia canis,* and *Ehrlichia ewingii*, in addition to antigen of adult *D. immitis* using the Idexx SNAP 4dx ELISA kit. This study assesses the species and burden of ectoparasites insects present on coyotes.

MATERIALS AND METHODS

Collection of Ectoparasites

Prior to making incisions for dissection, each coyote was examined closely for ectoparasites such as fleas, ticks, lice, and mites. A fine-toothed flea comb (Oster® wooden-handled flea comb) was used to remove small ectoparasites (fleas and lice) from the specimen's fur as well as to aid in the location of ticks, and forceps were used to remove ticks from the fur and skin. All ectoparasites located on each host were collectively placed in a vial containing 70% ethanol, labeled, and set aside for microscopic evaluation and species identification in the laboratory after dissection of the carcass concludes.

Evaluation of Ectoparasites

All ectoparasites were stored in 70% Ethanol after removal from the coyote and were then transported to the lab for identification under a microscope. "*A Key to the Lice of Man and Domestic Animals*" was used to identify louse present to species and sex (Tuff, 1977). "*Pictorial Key to the Adults of Hard Ticks, Family Ixodidae (Ixodida: Ixodoidea), East of the Mississippi River*" was used to identify all adult ticks to sex and species (Keirans & Litwak, 1989), and "*Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found in the United States*" was used to identify nymphal stages of ticks found (Kierans & Durden, 1998). "Fleas: Pictorial Guide to Some Common Species in the United States" was used for species identification of fleas (Fritz & Pratt, 1954).

A two-sample t-test was used to analyze differences in frequency of infestation in male coyotes compared to female coyotes. Analysis of Variance (ANOVA) was used to determine differences in frequency of infestation based on geographic and DNR management region.

Evaluation of blood samples

The sample of blood and/ or pleural fluid drawn from the chest cavity was used to test for arthropod borne diseases. A rapid-dot ELISA test called Idexx SNAP 4dx (Idexx Laboratories, Inc., Westbrook, Maine-LOT#DP859) was used to test for exposure to tick-borne disease-causing pathogens. The SNAP 4dx test

indicates exposure to *Anaplasma phagocytophilum*/*Anaplasma platys* (Anaplasmosis), *Borrelia burgdorferi* (Lyme disease), and *Ehrlichia canis*/*Ehrlichia ewingii* (Ehrlichiosis) by testing for the presence of antibodies against these pathogens. Additionally, the SNAP test also detects antigens secreted by adult *Dirofilaria immitis* (Canine heartworm). All SNAP 4dx tests were performed as directed on the provided instructional booklet- three drops of the blood sample and four drops of conjugate were mixed and added to the sample well. Once the sample reached the activation window, the device was activated and results were observed after the 8-minute waiting period. A positive or negative result was recorded for each of the 4 tests on the SNAP device.

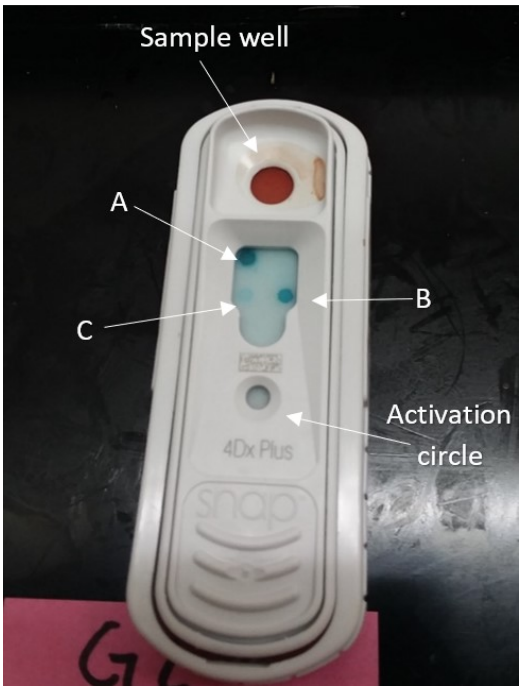

Figure 4.1: Idexx SNAP 4Dx test with positive control dot (A), positive *Dirofilaria immitis* result (B), and light positive result for *Ehrlichia canis* and or *Ehrlichia ewingii* antibodies (C).

RESULTS AND DISCUSSION

Results for ticks found on Canis latrans

A total of 128 ticks were collected from the 38 coyotes analyzed between December 2017 and March 2019, with 19 coyotes having at least one tick present (50% prevalence). *Amblyomma americanum* and *Ixodes scapularis* were collected most frequently, with *Amblyomma maculatum* and *Dermacentor variabilis* occurring in smaller numbers (Tables 4.1 & 4.2, Figures 4.2 & 4.3). Male coyotes were infested with ticks more frequently and at higher burdens than female coyotes. Ticks were found on 11 of 19 males and 8 of 19 females examined, and the average number of ticks on male coyotes was 4.6 compared to 2.1 in females (n=38, df=1, Levene p=0.0078). The number of ticks collected per coyote varied based on the geographic region that the coyote originated from (n=38, df=3, Levene p=0.0023), with the 5 most heavily infested coyotes all originating from the Piedmont region (Table 4.1; Figure 4.2). Number of ticks varied less significantly between DNR management regions (n=38, df=5, Levene p=0.0004) (Table 4.2; Figure 4.3)

Table 4.1: Species and quantity of ticks collected from coyotes by geographic region.

Geographic Region	**Species Found (Number)**	**Number of Coyotes Infested**	**Total Coyotes Tested**
Ridge and Valley	N/A	0	2
Piedmont	*Amblyomma americanum* (46) *Ixodes scapularis* (45)	9	19
Upper Coastal Plain	*Amblyomma americanum* (12) *Amblyomma maculatum* (7) *Dermacentor variabilis* (1) *Ixodes scapularis* (7)	8	13
Lower Coastal Plain	*Amblyomma americanum* (1) *Amblyomma maculatum* (1) *Ixodes scapularis* (8)	2	4

Table 4.2: Species and number of ticks collected from coyotes by DNR Management region.

DNR Region	Species Found (Number)	Number of Coyotes Infested	Total Coyotes Tested
Northeast	*Amblyomma americanum* (6)	1	6
Northwest	*Amblyomma americanum* (1)	1	6
East Central	*Amblyomma americanum* (3) *Ixodes scapularis* (43)	3	4
West Central	*Amblyomma americanum* (39) *Amblyomma maculatum* (1) *Ixodes scapularis* (5)	6	7
South Central	*Amblyomma americanum* (1) *Amblyomma maculatum* (7) *Ixodes scapularis* (11)	4	8
Southwest	*Amblyomma americanum* (9) *Dermacentor variabilis* (1) *Ixodes scapularis* (1)	4	7

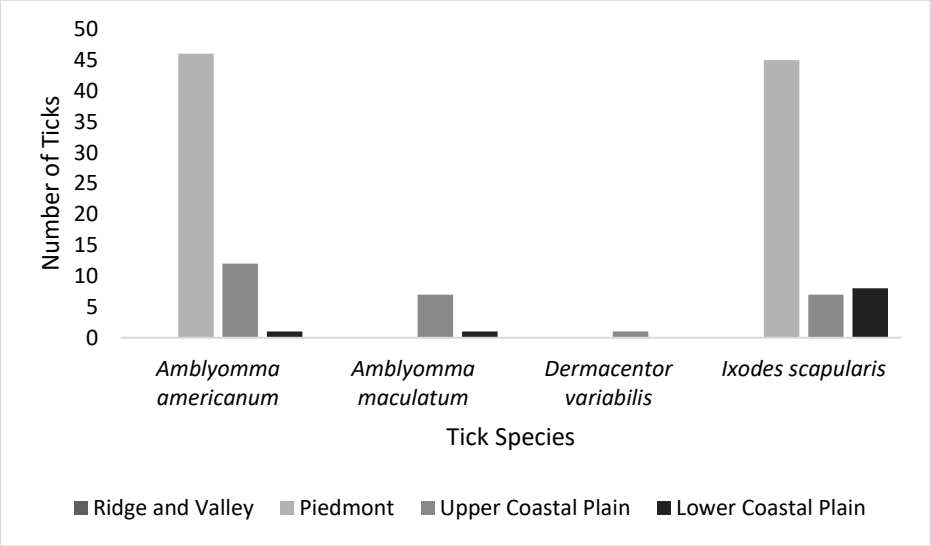

Figure 4.2: Number of ticks collected by geographic region.

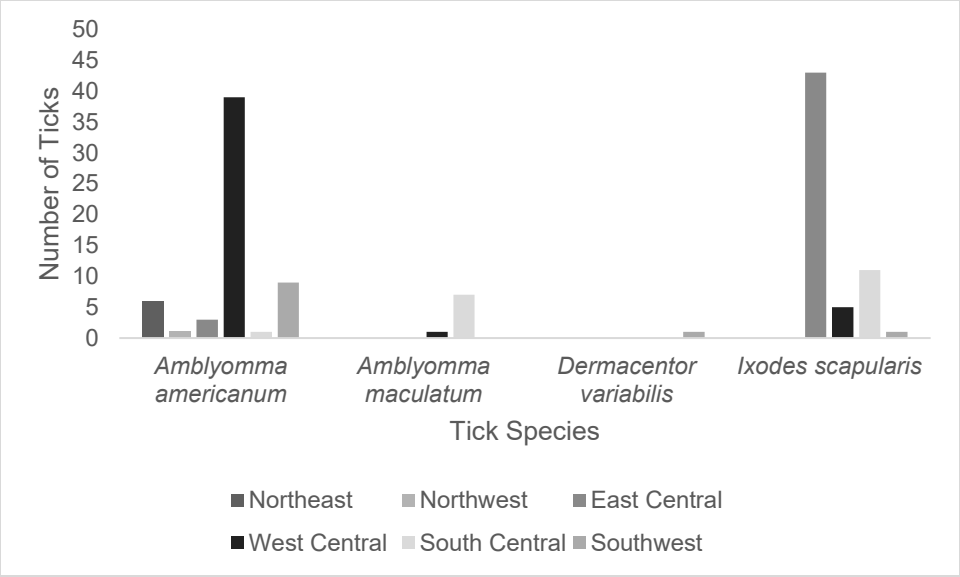

Figure 4.3: Number of ticks collected by DNR management region.

Results for ectoparasitic insects found on Canis latrans

Only 4 of the 38 coyotes were found to have fleas present (Table 4.3). Three of the four coyotes had single species infections, while one had two species of fleas present. All fleas were identified to species, with the exception of *Pulex* sp. found (all females), due to the inability to distinguish female *Pulex irritans* from *Pulex simulans* based on morphological characteristics alone. *Trichodectes canis*, the canine chewing louse, was found on 6 of the 38 coyotes examined (Table 4.4), with burden of lice collected per specimen varying greatly. All life stages and sexes of *Trichodectes canis* were found on coyotes with heavy infestations (Figure 4.4).

Table 4.3: Species, quantity, and origin of fleas found on 4 coyotes.

Date Collected	**Species and Quantity of flea**	**DNR Region**	**Geographic Region**
December 13, 2018	*Pulex* sp. (2)	South Central	Lower Coastal Plains
December 18, 2018	*Pulex* sp. (1)	East Central	Piedmont
December 19, 2018	*Ctenocephalides felis* (2)	East Central	Piedmont
December 19, 2018	*Pulex* sp. (1) *Cediopsylla simplex* (4)	East Central	Piedmont

Table 4.4: Number, sex, and origin of *Trichodectes canis* found on 6 coyotes.

Male *T. canis*	Female *T. canis*	Nymphal *T. canis*	DNR Region	Geographic Region
0	1	0	East Central	Piedmont
19	85	70	Northeast	Piedmont
9	6	12	Northwest	Piedmont
0	1	0	Northwest	Piedmont
19	29	4	South Central	Upper Coastal Plains

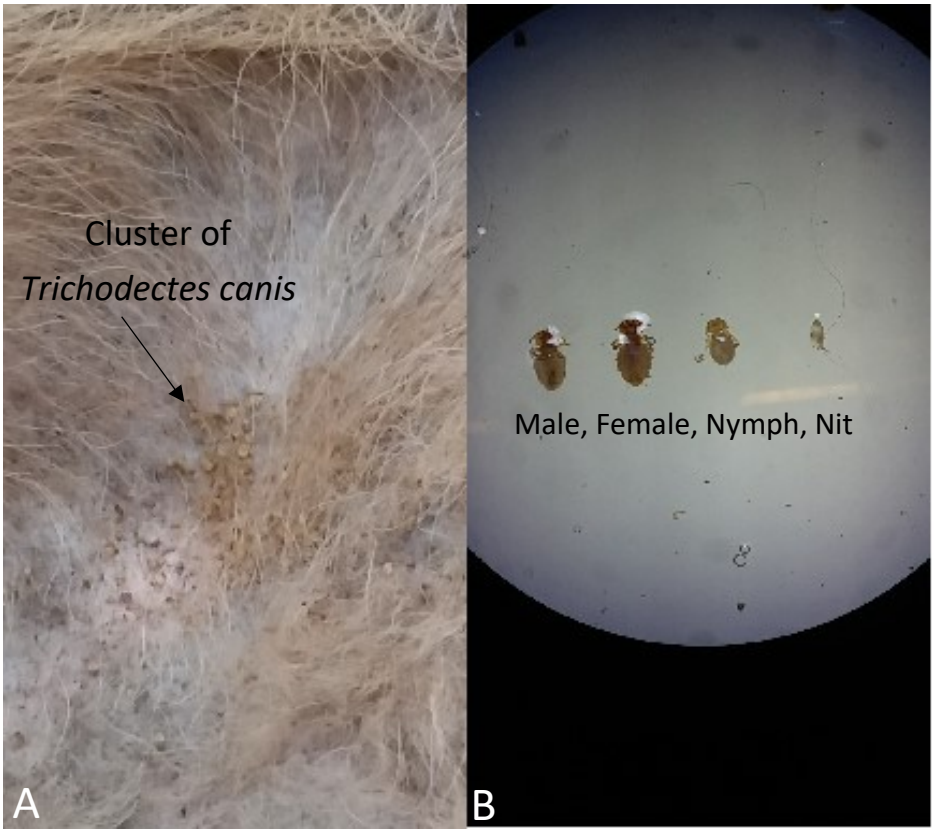

Figure 4.4: Infestation of *Trichodectes canis* found on *Canis latrans* from South Central/ Upper Coastal Plains region (A), microscopic examination revealed males, females, nymphs, and nits (eggs) (B).

Results for exposure to tick-borne illness found in Canis latrans

Of the 38 coyotes analyzed in this study, 30 were tested for exposure to three common tick-borne illnesses. 10 out of 30 (33.33%) of coyotes tested positive for exposure to some form of tick-borne illness. Exposure to *Ehrlichia* spp. (*E. canis* and/ or *E. ewingii*) was found in 10 coyotes, while exposure to *Borrelia burgdorferi* was only observed in 1 coyote, and exposure to *Anaplasma* sp (*A. platys* and/ or *A. phagocytophilum*). was not observed. The one coyote which tested positive for exposure to *Borrelia*

burgdorferi was also positive for exposure to *Ehrlichia* sp.. While coyotes from all geographic and DNR regions were tested (Table 4.5 & Table 4.6), the 3 tick borne illness tested for were not observed in coyotes from the Ridge and Valley/ Northwest region (Figure 4.5 & Figure 4.6).

Table 4.5: Results of antibody test (Idexx SNAP 4dx) for exposure to *Ehrlichia ewingii*/ *Ehrlichia canis, Borrelia burgdorferi, and Anaplasma platys/ Anaplasma phagocytophilum* by DNR Management region.

DNR Region	**Number Positive for *Ehrlichia* spp.**	**Number Positive for *Borrelia* spp.**	**Number Positive for *Anaplasma* spp.**	**Number tested**
Northeast	1	1	0	6
Northwest	0	0	0	4
East Central	3	0	0	4
West Central	2	0	0	5
South Central	4	0	0	8
Southwest	0	0	0	2

Table 4.6: Results of antibody test (Idexx SNAP 4dx) for exposure to *Ehrlichia ewingii*/ *Ehrlichia canis, Borrelia burgdorferi, and Anaplasma platys/ Anaplasma phagocytophilum* by geographic region.

Geographic region	**Number Positive for *Ehrlichia* spp.**	**Number Positive for *Borrelia* spp.**	**Number Positive for *Anaplasma* spp.**	**Number tested**
Ridge and Valley	0	0	0	2
Piedmont	6	1	0	15
Upper Coastal Plains	3	0	0	8
Lower Coastal Plains	1	0	0	4

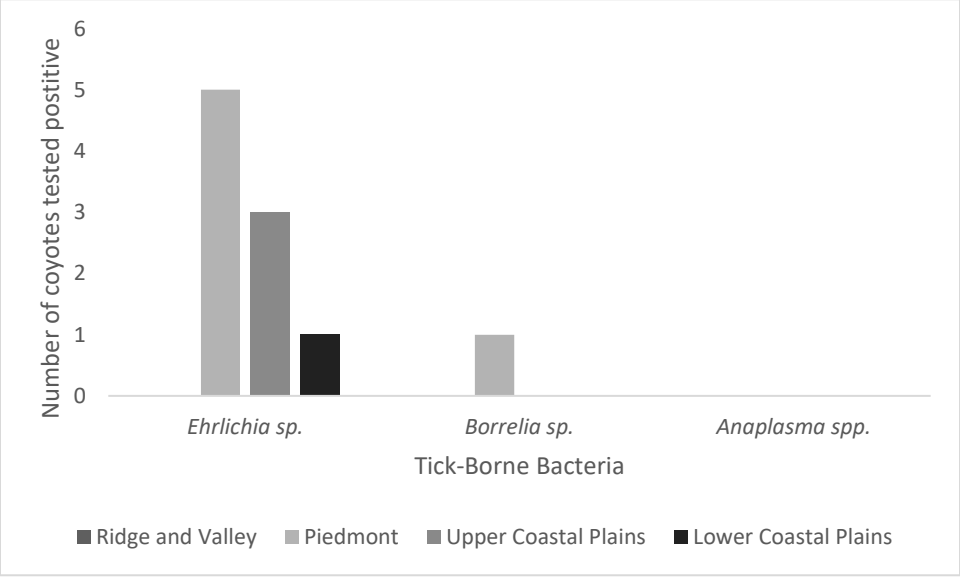

Figure 4.5: Coyote exposure to tick borne-illness by geographic region.

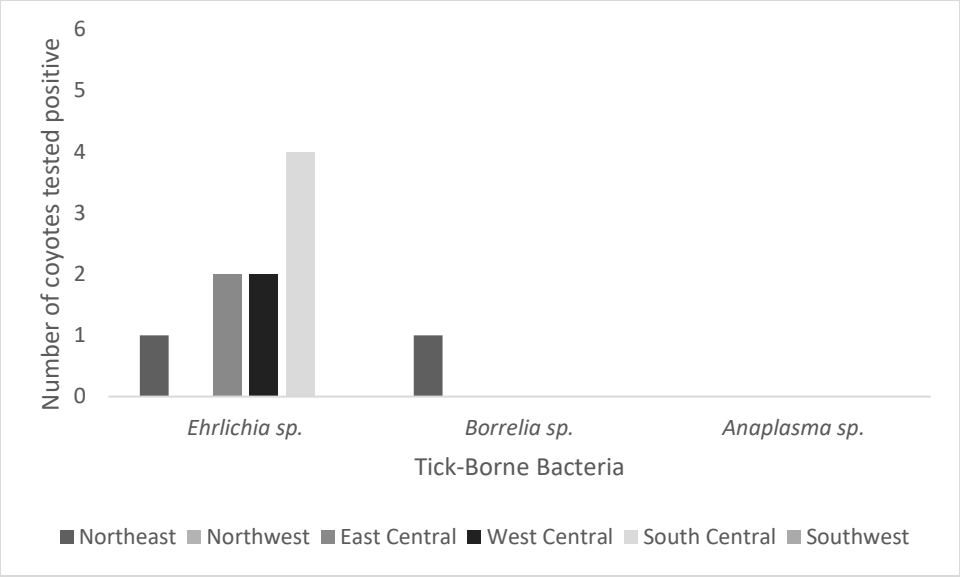

Figure 4.6: Coyote exposure to tick borne-illness by geographic region.

Infestation of Canis latrans with ticks

Exactly 50% of the coyotes examined in this study were found to be infested with ticks (n=38, 19 infested). *Ixodes scapularis* and *Amblyomma americanum* (Figure 4.7) were found most frequently, with 60 and 59 ticks collected respectively, while 8 *Amblyomma maculatum* and 1 *Dermacentor variabilis* were collected. The species of ticks collected were similar to those reported in recent studies of a study on coyotes in Georgia (Gates et al, 2014) and wildlife host-tick associations in Florida (Hertz et al, 2017), and . While the 50% observed prevalence of tick infestations is much lower than the 100% prevalence found in a similar study performed in Central GA, their immediate access to the coyotes after death likely allowed for a more accurate retrieval of ectoparasites (Gates et al, 2014). Despite a memo about the study taking place on coyotes entered in the competition and the importance of ectoparasite containment distributed to hunters prior to the event, coyotes from the Georgia Predator Hunting Association's "Coyote Challenge" in both 2018 and 2019 (n=21) were left un-bagged for up to 36 hours post-mortem before dissection began, and some individual hunters left the animals out for 2-3 hours before bagging. Three individual trappers with experience in wildlife research bagged their specimen immediately post-mortem, which resulted in a higher prevalence of ectoparasites (fleas, ticks, or lice) found on 9 of the 13

coyotes which they submitted (69%). One interesting trend was the number of sampled coyotes infested with ticks from the Coyote Challenge from 2018 to 2019. Hunting occurred February 23-24, 2018 with collection in Fort Valley, GA on February 25th, and 11 of 14 coyotes were found to be infested with ticks. The following year, hunting occurred from February 8-9 with collection on the 10th at the same location as 2018, and ticks were not found on any of the 7 coyotes sampled. The temperature was quite variable between the two years, ranging from 58°F to 85°F in areas hunted from February 23-34, 2018 and 33°F to 56°F in areas hunted from February 8-9, 2019 (Atlanta Weather Records Database, 2019). Additionally, all 4 coyotes collected 2 weeks prior (January 23-24, 2019) and submitted from a trapper who bagged immediately post-mortem were also not found to be infested with ticks. The temperature in the area where these coyotes were trapped ranged from 38°F to 39°F on the days these coyotes were trapped (Atlanta Weather Records Database, 2019). However, the lower temperatures are not likely to be the factor in the absence of ticks as tick infestations were present on all specimens submitted from the same trapper on December 18-19, 2018 when temperatures ranged from 36°F to 64°, and another trapper on December 07th and 13th, 2018 when temperatures ranged from 39°F to 59°F, and 39°F to 61°F, respectively. While ambient temperatures do have an effect on ticks and their abundance (Ogden et al, 2005), the mild temperatures during the winter season in Georgia are unlikely to have caused the trend of no ticks observed on specimen in January and February of 2019.

Several pairs of *Ixodes scapularis* ticks were removed from the coyotes with males and females in ventral contact (Figure 4.8). The orientation of the male's capitulum near the female's genital pore is consistent with insemination, or perhaps a sperm-guarding behavior to block the female's genital pore from any other males (Kiszewski et al, 2001). It has been observed that while ticks from the genus *Ixodes* require about 1 hour of genital contact for successful insemination, they can remain attached after mating for longer periods of time spanning from 2 hours to several days (Kiszewski et al, 2001).

Figure 4.7: *Amblyomma americanum* females at various stages of engorgement removed from a single coyote.

Figure 4.8: Male (smaller) and female (larger) *Ixodes scapularis* ticks removed from coyote.

Infestation of Canis latrans with ectoparasitic insects

Unfortunately, collection of fleas from coyotes was not highly successful, with fleas collected from only 4 coyotes. All coyotes from which fleas were collected originated from 2 trappers who bagged coyotes immediately post-mortem, which likely contributed to the success in finding fleas as they were contained. Fleas are capable of leaving a host, and do so frequently after a host dies and the body temperature begins to drop (Personal Experience from Veterinary Work). Therefore, it is likely that infestation with fleas in coyotes is much more prevalent than that found in this study.

Trichodectes canis, the Canine Chewing Louse, was found in varying burdens on 6 of the 38 examined coyotes- the number of lice collected ranged from 1 to 174 per specimen. Immediate bagging of the

coyote post-mortem did not have an effect on whether or not lice were found, as 33% of coyotes found to have lice infestations were examined after being left exposed to the elements for 24-48 hours post-mortem. In cases in which the coyote was bagged post-mortem, the lice remained in place and were not crawling around on the carcass in efforts to leave the host as fleas were. Because *Trichodectes canis,* an intermediate host for the tapeworm *Dipylidium caninum* (Roberts et al Ch. 36, 2013), can also affect domestic canines, a risk for domestic dogs to contract tapeworms by ingesting a louse while grooming or feeding on a coyote carcass exists. Additionally, there is a possibility for domestic dogs in close contact with coyotes to contract lice by close physical contact; however, the likelihood of domestic dogs being affected by *Trichodectes canis* residing on coyotes is very small.

Exposure of Canis latrans to tick-borne diseases

The 38 coyotes which were tested for exposure to tick-borne diseases were chosen based on availability of an anticoagulated sample of blood or pleural fluid. The ELISA test kits used in this experiment (Idexx SNAP 4dx) are used in Veterinary clinics to assess exposure to the tick-borne pathogenic bacteria that cause Anaplasmosis (*Anaplasma phagocytophilum* and *Anaplasma platys),* Lyme disease *(Borrelia burgdorferi),* and Ehrlichiosis *(Ehrlichia canis* and *Ehrlichia ewingii),* and were chosen based on availability, accuracy, ease of use, cost-effectiveness, and small volume of blood or serum needed (3 drops). Exposure to all 3 diseases is measured on a testing device with 3 separate areas, each of which indicates the presence or absence of antibodies against the bacteria in question in the animal's blood. The SNAP 4dx test is effective in measuring exposure only, and therefore does not differentiate between a canine harboring an active infection and a canine which was exposed but did not develop an infection (Idexx, 2019).

Only 2 of the 10 coyotes which had been exposed to *Ehrlichia* spp. were found to have been infested with *Amblyomma americanum*, the known vector of *Ehrlichia ewingii* on them at the time of dissection,

and the 1 coyote exposed to *Borrelia burgdorferi* did not have *Ixodes scapularis* (vector) present when dissected, which indicates that the current absence of a tick infestation on an animal is not indicative of past infestations and exposure to disease. An interesting direction for future experimentation would be to further analyze the burden of tick-borne illnesses of veterinary and medical importance in Georgia's coyote populations by obtaining fresh, anticoagulated blood, performing ELISA tests for pathogens of interest, and then running titers on any coyote with a positive ELISA result to assess whether or not the coyote is harboring an active infection based on the antibody titers present in the blood. Due to the absence of exposure to *Anaplasma* sp. bacteria in this study's sample population, and the extremely low incidence of *Anaplasmosis* exposure in the Southeast (0.5%) compared to the national mean of 4.8% (Bowman et al, 2008), it may be practical to discontinue Anaplasmosis screening and test for exposure to Lyme disease (*Borrelia burgdorferi*) and Ehrlichiosis (*Ehrlichia canis* and *Ehrlichia ewingii*). Because human Spotted Fever Rickettsioses are more prevalent in the Southeast (Biggs et al, 2016), testing for *Rickettsia* spp. bacteria such as *Rickettsia rickettsii* or *Rickettsia parkeri* in the local coyote populations may be more practical than testing for exposure to Anaplasmosis. In conclusion, coyotes pose a risk to domestic canines and humans by acting as a reservoir of tick-borne pathogenic bacteria such as *Borrelia burgdorferi and Ehrlichia* spp., and by acting as a mode of transportation for ticks. While exposure to the following pathogens were not tested in this study, the presence of their vectors on the examined coyotes indicates the risk of coyotes as a mode of transportation for: *Ehrlichia chaffeensis, Francisella tularensis,* Heartland Virus, and the bacterium responsible for STARI (Vector *Amblyomma americanum*); *Rickettsia parkeri* (Vector *Amblyomma maculatum*); and *Rickettsia rickettsii* (Vector *Dermacentor variabilis*).

CONCLUSIONS

In a study of 38 coyotes collected throughout Georgia, USA, a total of 1086 parasites were collected and identified: 215 intestinal nematodes, 116 cestodes, 1 acanthocephalan, 359 filarial nematodes, 128 ticks, 10 fleas, and 257 lice. Three specimens had no parasites of any form present (7.89%), while 11 of the specimens were found to be affected by heartworms, intestinal parasites, and ectoparasites (28.94%). One specimen was positive for all classifications of parasites tested for in this study, including *Dirofilaria immitis, Taenia* spp., *Amblyomma americanum, Ixodes scapularis, Ctenocephalides felis,* and *Trichodectes canis.* Of 30 coyotes analyzed for exposure to tick-borne illness, 10 were found to have been exposed to *Ehrlichia canis* or *Ehrlichia ewingii* (33%), and 1 was found to have been exposed to *Borrelia burgdorferi* (3.33%).

Reducing parasitism in Georgia's coyote population is a virtually impossible task. Performing routine prophylaxis for helminths and ectoparasites is difficult in free-roaming animals, and leaving out medicated treats or application stations could pose a risk to other animals which may encounter them. Additionally, the approximate population of coyotes in Georgia (>300,000) makes treatment even more difficult and unrealistic. The information found in this study on parasites of coyotes supports their role as a wildlife reservoir host for parasites and various arthropod-borne diseases. With coyotes expansion into more suburban and urban landscapes, the results of this study are important to inform Georgians of the risks associated with the presence of coyotes near their residences and precautions to take to protect themselves and their pets, as the coyotes examined in this study were found to be a host and mode of transportation for parasites which can negatively affect the health of both humans and domestic animals.

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